PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

B10

(51) International Patent Classification 6 :
A61K 47/38, C08B 15/00, 31/00, A61L 29/00

A2

(11) International Publication Number: WO 96/02276

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/US95/09815

(22) International Filing Date: 18 July 1995 (18.07.95)

(30) Priority Data:

08/276,193	18 July 1994 (18.07.94)	US
08/413,409	30 March 1995 (30.03.95)	US

(71) Applicant: GEL SCIENCES, INC. [US/US]; 213 Burlington Road, Bedford, MA 01730 (US).

(72) Inventors: SCHILLER, Matthew, E.; 23C Sagamore Way, Waltham, MA 02154 (US). GEHRKE, Stevin, Henry; 5444 Sprucewood Drive, Cincinnati, OH 45239 (US). LUPTON, Elmer, C.; 23 Pinckney Street, Boston, MA 02114 (US). TANAKA, Toyoichi; 22 Lowell Road, Wellesley, MA 02181 (US). YU, Xiaohong; Apartment 805, 130 Bowdoin Street, Boston, MA 02108 (US).

(74) Agent: JARRELL, Brenda, H.; Choate, Hall & Stewart, 53 State Street, Exchange Place, Boston, MA 02109 (US).

(81) Designated States: AU, CA, CN, FI, JP, KR, MX, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: NOVEL POLYMER GEL NETWORKS AND METHODS OF USE

(57) Abstract

A cross-linked, responsive polymer gel network comprising polymer chains interconnected by way of a multifunctional cross-linker is described. The polymer chains and cross-linker have a known acceptable toxicological profile (KATP). Pathways for determining if a particular material has a KATP are provided. The polymer chains should be a component part of a product that is considered in compliance with applicable governmental agency regulations as acceptable for a use selected from the group consisting of a food use, a cosmetic use, and an animal drug delivery use. Moreover, the cross-linker also should be a component part of a product that is considered in compliance with applicable governmental agency regulations as acceptable for a use selected from the group consisting of a food use, a cosmetic use, and an animal drug delivery use.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria
AU	Australia
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CS	Czechoslovakia
CZ	Czech Republic
DE	Germany
DK	Denmark
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgystan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakhstan
LI	Liechtenstein
LK	Sri Lanka
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SI	Slovenia
SK	Slovakia
SN	Senegal
TD	Chad
TG	Togo
TJ	Tajikistan
TT	Trinidad and Tobago
UA	Ukraine
US	United States of America
UZ	Uzbekistan
VN	Viet Nam

NOVEL POLYMER GEL NETWORKS AND METHODS OF USE

This application is a Continuation-in-part of co-pending application Serial Number 08/276,193, filed July 18, 1994, the entire contents of which are hereby incorporated by reference.

Background of the Invention

Volumetric change phenomena have been observed in three-dimensional, permanently crosslinked polymer gel networks. As an external environmental condition (e.g. temperature; solvent composition; pH, electric field; light intensity and wavelength; pressure, ionic strength) is changed, the polymer gel network contracts and/or expands in volume. The volume of such a gel may, under certain circumstances, change reversibly by a factor as large as several hundred when the gel is presented with a change in external conditions (i.e. the gel is a "responsive" gel; see, for example, Tanaka Phys. Rev. Lett. 40: 820, 1978; Tanaka et al. Phys. Rev. Lett. 38:771, 1977; Tanaka et al. Phys. Rev. Lett. 45:1636, 1980; Ilavsky Macromolec. 15:782, 1982; Hrouz et al. Europ. Polym. J. Vol. 17, pg. 361, 1981; Ohmine et al. J. Chem. Phys. 8:6379, 1984; Tanaka et al. Science 218:462, 1982; Ilavsky et al. Polymer Bull. 7:107, 1982; Gehrke "Responsive Gels:Volume Transitions II", Dusek (ed.), Springer-Verlag, New York, pp. 81-144, 1993; Li et al., Ann. Rev. Mat. Sci. 22:243, 1992; and Galaev et al. Enzyme Microb. Technol. 15: 354, 1993, each of which is incorporated herein by reference).

A number of significant studies have demonstrated the potential of responsive gels in solute/solvent separations (see, for example, Cussler U.S. Patent 4,555,344) and in biomedical applications (see, for example, Hoffman U.S. Patent 4,912,032). In spite of this, responsive gels have failed to become commercially useful for two major reasons. Synthesis of a gel may utilize monomers and/or polymers whose toxicologic hazard characteristics are ill-defined (e.g. N-isopropylacrylamide (NIPA) and related acrylic monomers, polymers and co-polymers). Second, synthesis of a gel may use crosslinkers known to be toxic

(e.g. divinyl sulfone (DVS), glutaraldehyde, divinyl benzene, N-N-methylenebisacrylamide, and the like).

Harsh and Gehrke (J. Control. Rel. 17:175, 1991, incorporated herein by reference) have created certain gels based on cellulose ether polymeric precursor materials. These cellulosic ether precursor materials are currently acceptable by the U.S. Food and Drug Administration, but these gels were made using toxic DVS crosslinkers that are not FDA acceptable. One way to avoid use of toxic chemical crosslinkers is by use of radiation crosslinking. This method is problematic inasmuch as it may lead to the presence of unreacted monomers.

While it is certainly possible that currently available, chemically crosslinked gel materials can prove to be biologically compatible for *in vivo* use (see, for example, Peppas et al. (eds) "Hydrogels in Medicine and Pharmacy" Vol. 1 and 2, CRC Press, Boca Raton, Florida (1986), the existing regulatory environment and the myriad of tests required to characterize the toxicity of such materials place major barriers to commercialization of responsive gels.

Summary of the Invention

It is an object of the present invention to provide responsive polymer gels that are environmentally safe and safe for use in humans.

It is another object of the invention to provide methods for making crosslinked, responsive polymer gels in which the crosslinker and polymer are acceptable to governmental regulatory agencies as safe for use in humans.

Yet another object of the invention is to provide improved controlled-release delivery systems in which safe, responsive gels are utilized to deliver a substance to an environment.

One embodiment of the invention is a crosslinked, responsive polymer gel network comprising polymer chains interconnected by way of multifunctional crosslinker. The polymer chains and crosslinker have a known acceptable toxicological profile, hereinafter "KATP". Another embodiment is a crosslinked, responsive polymer gel network comprising polymer chains interconnected by way of a KATP crosslinkages. A third embodiment of the invention is a

crosslinked, responsive polymer gel network comprising polymer chains interconnected by way of a crosslinker, in which each of the polymer and crosslinker is obtainable from a precursor that is used in a process for making a material that has a KATP. The gels have the characteristic that, when leached, the leachate from the network also has a KATP as well as any residual elements in the network. The gel solvent also may have a KATP.

A preferred responsive polymer gel network are polysaccharide chains crosslinked with a multifunctional carboxylic acid obtainable from an acyl halide derivative of said acid. The preferred polymer chains are polysaccharides (e.g. starch or cellulose ethers) and the preferred multifunctional carboxylic acid is selected from the group consisting of adipic acid, sebacic acid, succinic acid, citric acid,1,2,3,4- butanetetracarboxylic acid, and 1,10 decanedicarboxylic acid. Particularly preferred polymers are cellulose ethers selected from the group consisting of hydroxyethylcellulose, hydroxypropylcellulose, and hydroxypropylmethylcellulose.

The responsive polymer gel networks of the present invention may be responsive to any of a variety of triggers such as temperature or pH. In particular, the pH-response may be triggered by a change in ion concentration, solvent concentration, electric field, magnetic field, electromagnetic radiation, or mechanical force.

Methods for making crosslinked polymer networks include selecting a polymeric starting material capable of being crosslinked, wherein the polymeric starting material selected for the particular use has a known acceptable toxicological profile for the particular use or for a related use; selecting a crosslinker capable of crosslinking the polymeric starting material, wherein the crosslinker selected for the particular use has a known acceptable toxicological profile for the particular use or for a related use; and contacting the crosslinker and polymeric starting material under conditions sufficient to form the three-dimensional, crosslinked polymer network.

Another method involves selecting a crosslinker capable of crosslinking the polymeric starting material, so that the resulting network, after formation, contains

a crosslinkage that has a known acceptable toxicological profile. Preferred methods include the steps of contacting a crosslinker comprising an acyl halide derivative of a multifunctional carboxylic acid with a polysaccharide under conditions sufficient for the three-dimensional, polymer gel network to form so that the gel network includes polysaccharide chains crosslinked with the acid. Particularly preferred method use a polysaccharide selected from the group consisting of starch and cellulose ethers, which group includes, for example, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, and modified food starch.

Preferred methods use a crosslinker that is an acyl halide derivative of a multifunctional carboxylic acid, such as, for example, an acyl halide derivative of adipic acid, sebacic acid, succinic acid, 1,2,3,4- butanetetracarboxylic acid, or 1,10 decanedicarboxylic acid. Other preferred methods use dialdehyde crosslinkers such as, for example, glutaraldehyde. Still other preferred methods utilize irradiation energy as a crosslinker.

Other methods of the invention include a method of separating a substance from a solution. A KATP polymer gel network of the invention capable of incorporating the substance from a solution containing the substance is introduced into the solution and a volumetric change of the gel is induced by changing an environmental condition to which the gel is exposed so that the gel incorporates the substance and separates the substance from the solution. A further method includes introducing a KATP polymer gel network of the invention that is capable of excluding a substance from a solution containing the substance. The gel is induced to undergo a volumetric change by changing an environmental condition to which the network is exposed so that the network disgorges the substance to the environment of use.

A method of delivering a substance into an environment of use includes the steps of incorporating the substance into the KATP polymer network of the invention and inducing a volumetric change in the network by changing an environmental condition to which the network is exposed so that the network disgorges the substance to the environment of use.

Similarly, a method for removing a substance from an environment containing a the substance includes introducing into the environment a KATP polymer network of the invention that contains a ligand reactive with the substance when the ligand is exposed to the substance and changing an environmental condition of the network to cause a volumetric change and expose the ligand to the substance, so that the substance is incorporated into the gel.

A method of loading a solute into a KATP polymer gel network includes contacting the solute with the KATP gel, a second polymer, and a salt under conditions sufficient for the solute to selectively partition into the first polymer.

A cosmetic composition, wound dressing, pharmaceutical composition, monitoring electrode; adhesive device, iontophoretic device; dialysis device; including the KATP polymer network are also intended to be encompassed within the scope of the invention.

The KATP responsive gel networks of the invention have the singular advantage of having toxicological profiles which are more readily evaluated than prior art responsive gels. Thus, the present responsive gels may be used as environmentally benign materials which may be easily recycled for many commercial purposes and which may be used in the human body, or in the body of a non-human animal. The present invention therefore provides novel pharmaceutical compositions, comprising a KATP polymer gel network (also termed a "safe" polymer gel network) and an effective amount of a biologically active compound. The present invention also provides controlled-release delivery devices for controlled delivery of a biologically active compound into a biological environment.

The KATP polymer gel networks of the present invention are also useful for coating materials, such as medical devices, and the invention encompasses such coated devices. In preferred embodiments of the coated medical devices of the present invention, a polymer gel network comprising a polycation and a polyanion interacting to form a polyelectrolyte complex is formed on at least a portion of a surface of the device. Biologically active compounds can be loaded into such coated devices, and in some embodiments an encapsulator is provided that traps

the biologically active compound within the polymer gel network. When the encapsulator comprises a KATP polymer, the invention essentially provides a safe polymer gel network coating on an existing safe polymer gel network. Methods of coating medical instruments, and of loading biologically active compounds into or delivering biologically active compounds from, such coated instruments, are also provided.

Brief Description of the Drawing

Various novel features of the invention, both as to its structure and operation, are best understood from the accompanying drawings, taken in conjunction with the accompanying description, in which similar reference characters refer to similar elements, and in which:

Figure 1 is a pH-volume relationship for a crosslinked HPC gel of the invention.

Figure 2 is a pH-volume relationship for a crosslinked modified food starch gel of the invention.

Figure 3, panels A-C, presents depictions of present-day osmotically-controlled oral delivery systems.

Figure 4 depicts formation of a safe polymer gel comprising polyelectrolyte complexes on a polyamide device.

Figure 5 depicts radiation-induced grafting of a safe polymer gel comprising polyelectrolyte complexes onto low density polyethylene.

Figure 6 depicts coating of a safe polymer gel comprising polyelectrolyte complexes onto a porous support.

Figure 7 depicts coating of a safe polymer gel comprising polyelectrolyte complexes onto a polypropylene membrane.

Detailed Description of Preferred Embodiments of the Invention

TABLE OF CONTENTS:

I. Morphology, definitions

II. Design Pathways

III. Starting Materials

A. Polymer

B. Crosslinker

C. Functional Groups

D. Additives

IV. Responsive Gel Networks

V. Methods of Preparing Polymer Networks

A. General Considerations

B. Microporous Gels

VI. Biologically Active Compounds

VII. Utilities/Formulations

A. Separation of a Solvent from a Solute

B. Separation of a Solute from a Solvent

i. Lipid Scavenging

C. Protection of a Compound's Activity

D. Delivery of a Compound into an Environment

i. Oral Delivery Formulations

ii. Mucosal Delivery Formulations

iii. Ocular Delivery Formulations

iv. Vaginal Delivery Formulations

v. Rectal Delivery Formulations

vi. Dermal Delivery Formulations

vii. Internal Delivery Formulations

viii. Agricultural Delivery Formulations

ix. Medical Instruments

ix. Cell Culturing Formulations

E. Pharmaceutical Compositions

F. Cosmetic Compositions

VIII. Examples

I. Morphology, definitions

The materials of the present invention are three-dimensional, permanently crosslinked polymer networks. As defined herein, a "polymer network" is that three-dimensional structure resulting from the crosslinking of polymers. Preferred polymers are chemically-crosslinked. "Chemically crosslinked" means that a multifunctional chemical reagent is added during synthesis which reacts with, and interconnects via covalent bonding, two or more polymer chains. The term "chemically crosslinked" is not meant to include gamma radiation, photochemical, electron beam, or ultraviolet crosslinking when these methods are intended to replace a chemical moiety used as a crosslinker. Thus, direct crosslinking of hydroxypropyl cellulose with itself using radiation is not intended to be included within the scope of this invention.

The multifunctional chemical reagent is the "crosslinker". The form that the crosslinker takes once the polymer network has been formed is defined as the "crosslinkage". For example, and as discussed more fully below, succinyl chloride is a crosslinker that may not have a KATP but, once it is reacted in solution and crosslinked, it converts to succinic acid crosslinkage which does have a KATP. It will be understood by those having ordinary skill in the art that, not every single crosslinking reaction will produce the desired KATP crosslinkage; some defects may arise. Nevertheless, within the constraints of good manufacturing practice for materials of this type that are well known to those of ordinary skill in the art, it is considered that gels of the present invention have crosslinkages that all have a KATP, although the starting material crosslinker (e.g. precursor acyl halide derivative) may not have a KATP.

Likewise, the polymer chains themselves may have impurities or other imperfections that are considered to be within tolerance requirements for materials of this type when used for the particular purposes of the present invention. Similarly, within the constraints of good manufacturing practice for materials of

this type that are well known to those of ordinary skill in the art, it is considered that gels of the present invention all have a KATP.

In particular, the preferred three-dimensional polymer networks are homogeneous or microporous gels. The term "gel" refers to materials between the liquid and solid state containing enough solvent molecules to cause macroscopic changes in the sample dimension. The term "gel" also includes polymer gel networks in which virtually all liquid (i.e., solvent) has been driven off, leaving a "dry" gel. The term "microporous" refers to two-phase systems of a continuous solid phase containing numerous pores filled with fluid. A "microstructure" as defined herein, refers to those structures of a gel (e.g. pores, voids, walls and the like) observable under a scanning electron, or other, microscope and ranging in size from 0.01 to about 100 microns. Gels containing pores in the size range 0.01 to about 10 microns are 'microporous'. If some of the pores are interconnected, the gel is typically called an "open-cell" gel. If all the pores in the gel are interconnected to each other, the gel is a "bicontinuous" gel. If the pores are discrete (not connected to each other), so that the internal space of each pore is independent of the other pores, the gel is a "closed-cell" gel. The present invention encompasses as all these morphological forms and combinations of these forms.

Microporous responsive gels may be "fast response" gels. As defined herein, "fast response" means that the gel reaches 90% of its maximum volumetric swelling or 90% of its minimum volumetric collapse in a time that is at least ten times faster than a comparable non-porous gel of the same geometry when both gels are subjected to a similar change in an environmental condition.

The polymer gel network materials described herein may also be employed in a variety of forms. For example, the materials may be used as films or membranes, tubes, hollow fibers, solid fibers, molded objects, solid particles, capsules, micelles or liposome-like structures. They may also be applied as coatings on solid surfaces (e.g. catheter tips) or in the pores of porous solids, as solutions, particulate suspensions and the like. Coatings may be applied to and/or attached to polymers, metals, ceramics, glasses, carbons and the like.

II. Design Pathways

The polymer gel networks of the present invention and their component parts are intended to have a known acceptable toxicological profile (hereinafter "KATP"). This term refers to the ability of a given polymer gel network or component thereof to successfully pass regulatory approval by an appropriate governmental body or industry group responsible for the safety of drugs, cosmetics, medical devices, pharmaceuticals, food additives, food processing and the like when the drugs, cosmetics, medical devices, pharmaceuticals, food additives, food processing and the like, are used in animals, including humans.

More particularly, and at least in the United States, a polymer network of the present invention is considered to have a KATP if the network, and its polymer/crosslinker components, are considered by the U.S. Food and Drug Administration ("U.S. FDA") to be safe for a particular use as cited in Section 21 of the Code of Federal Regulations (" 21 C.F.R."), relevant sections of which are described below and incorporated herein by reference. Moreover, certain industrial groups have also provided lists of materials that are intended to be included within the scope of this invention, such groups in the United States including the Cosmetic,Toiletries and Fragrances Association (hereinafter "CTFA").

It will be understood that the U.S. Food and Drug Administration will only approve materials for a specific use and for a particular drug and dosage at issue. For example, materials that are considered to have an KATP suitable for the present invention include those on the "Generally Regarded as Safe" (GRAS) list promulgated by the Food and Drug Administration at 21 C.F.R.182.1-182.8997, when used for the purpose indicated in accordance with good manufacturing practices. GRAS materials useful in forming crosslinked polymer networks of the invention include methylcellulose (21 C.F.R. 182.1480); adipic acid (21 C.F.R. 184.1009) and succinic acid (21 C.F.R. 184.1091). Other materials that are considered to have a KATP are those that are permitted for direct consumption as food additives (amino acids- 21 C.F.R. 172.320), or as binders/fillers, film forming agents and thickeners for food such as ethylcellulose (21 C.F.R.

172.868); hydroxypropylcellulose (21 C.F.R. 172.870); methyl ethylcellulose (21 C.F.R. 172.872); and hydroxypropylmethylcellulose (21 C.F.R. 172.874); modified food starch (21 C.F.R. 172.892).

Materials suitable for treating, processing, or packaging food are also considered to have a KATP under the design pathways described herein. Exemplary substances include: polyvinylpyrrolidone (21 C.F.R. 173.55); adipic acid, fumaric acid, sebacic acid, and maleic acid (21 C.F.R. 175.300 (b) (vii) (a)) ; carboxymethylcellulose, ethylcellulose, ethyl hydroxyethylcellulose, hydroxypropylmethylcellulose, and methyl cellulose (21 C.F.R. 175.300 (b) (xvi)).

It will also be understood that the polymer gel in its crosslinked form must have an aqueous leachate that has a KATP or the aqueous leachate must contain residual moieties (i.e. materials left over from synthesis) such that the solvent is within acceptable limits promulgated by the appropriate regulatory body such as the U.S. FDA. FDA test procedures for determining leachates and residuals from polymeric coatings may be found in 21 C.F.R. 175.300 (Table 2).

Other materials that can functionalize the polymers of the invention and are considered to have a KATP under the pathways described herein are surfactants such as diethanolamide condensates, n-alkyl (C_8-C_{18}) amine acetates, and di-n-alkyl (C_8-C_{18}) dimethyl ammonium chloride (21 C.F.R. 172.710).

From the above-described considerations, we have developed a number of design pathways to allow persons having ordinary skill in the art to select materials which have a KATP and which are suitable as building block precursors of the polymer networks described herein:

Pathway Number 1: The individual polymer precursor(s), the crosslinker used in the synthesis, the final three-dimensional polymer network that is to be used in a particular context (e.g. food separation, drug release, medical devices, cosmetics) and, optionally, the liquid solvent incorporated within a polymer network, must all have a KATP for that particular use or must have a KATP for a related use. Less preferred, although still acceptable under Pathway 1, is the

corollary that the crosslinked polymer, as well as the final three-dimensional polymer network that is to be used in a particular use context, e.g. food separations, medical devices, drug release, cosmetics), have a KATP suitable for a different use.

Pathway Number 2: This pathway concerns the methods for making desirable KATP polymer gels. The polymer and crosslinker precursors and any processing aids (i.e. surfactants, anti-foaming compounds, and the like) used in synthesis of a KATP gel must also be used in processes for making other materials that have a KATP for the same, or for a related use. Less preferred, although still acceptable under Pathway 2, is the corollary that the precursors and aids are used in other processes for making materials that have a KATP for a different use.

A polymer network falling within any single pathway, or any combination of pathways, is suitable and is intended to be encompassed within the scope of the invention.

What follows are some examples, by no means exhaustive, of the applications of these heuristics:

1. One would like to develop a gel of the invention suitable for use in food separations. It is known that cellulose ethers (i.e., hydroxypropylcellulose-HPC) are suitable as polymer backbones since they have a KATP when used for food additives (See Aqualon Product Data Sheet No. 494.3, Aqualon Company, Little Falls Centre One, 2711 Centreville Road, PO Box 15417, Wilmington, DE 19850-5417). Cellulose ethers may be crosslinked with adipic acid and both HPC and adipic acid have KATP's for use as food additives (21 C.F.R. 172.870), thus satisfying Pathway 1.

Moreover, adipoyl chloride may be used in the synthesis of the adipic acid crosslinkage (see Pine et al. *Organic Chemistry*, McGraw-Hill, p. 319, 1980 and Example 1, *infra*). Adipoyl chloride is used in the production of commercial penicillins (see, for example, Laubeck et al. J. Chrom. Sci. 14, 1976), which is consistent with Pathway 2. Further, an aqueous leachate from a polymer gel

consisting of HPC crosslinked with adipic acid would be adipic acid and/or the cellulose ether (both KATP materials).

2. One would like to develop a gel of the invention suitable for use in parenteral drug release (see, for example, Siegel et al. *J.* Contr. Release 8: 179, 1988; Hoffman Controlled Release 6: 297, 1987). Similar considerations apply to developing suitable pathways for selection of these materials. Cellulose ethers (i.e., hydroxypropylcellulose-HPC) are suitable as polymer backbones since they have a KATP when used for food additives (Aqualon Product Data, supra). Cellulose ethers may be crosslinked with adipic acid and both HPC and adipic acid have KATP's for use as food additives (see above), thus satisfying Pathway 1. Moreover, adipoyl chloride may be used in the synthesis of the adipic acid crosslinkage (see above), thus satisfying Pathway 2. Further, an aqueous leachate from a polymer gel consisting of HPC crosslinked with adipic acid would be adipic acid and/or cellulose ether.

3. One would like to develop a pH-sensitive polymer gel suitable for use in agricultural controlled release. Cellulose ethers (i.e. hydroxypropylcellulose-HPC) are suitable as polymer backbones since they have a KATP when used for food additives (Aqualon Product Data, supra). Cellulose ethers may be crosslinked with adipic acid and both HPC and adipic acid have KATP's for use as food additives (see above), thus satisfying pathway 1. Surfactants such as diethanolamide condensates, n-alkyl ($-C_8$ $-C_{18}$) amine acetates, and di-n-alkyl (C_8-C_{18}) dimethyl ammonium chloride (21 C.F.R. 172.710), suitable for use with pesticides, may be added to the gel, satisfying Pathway 2 that starting materials may be used in processes for making other KATP materials.

Persons having ordinary skill in the art may readily determine if a particular material is suitable for use in the polymer networks of the present invention. Such persons need access to governmental regulatory agency rules that describe acceptable materials for a particular use (see above-cited FDA regulations). Those having ordinary skill in the art will readily appreciate that

specific materials considered to have a KATP will not necessarily be the same among countries having governmental agencies analogous to the U.S. FDA. Nevertheless, such persons will be able to ascertain which materials would have a KATP suitable for use in foreign countries by contacting the relevant, counterpart agency in that country. For example: in France the counterpart to the U.S. FDA is the Agence Francaise du Medicament- 143-145, boulevard Anatole France- 93200 Saint Denis; in the United Kingdom, the counterpart agency is the Medicines Control Agency, Market Towers, London, SW8 5NL; in Japan, the counterpart agency is the Ministry of Health and Welfare (drugs and devices for human use) and the Ministry of Agriculture and Fisheries (drugs and devices for non-human use); in Canada, the counterpart agency is the Drug Regulatory Affairs Division (drugs and devices for human use) and the Department of Agriculture (drugs and devices for veterinary use); in Germany, the counterpart agency is the Bundesgesundheitsamt, Institut fur Arzneimittel, Berlin.

III. Starting Materials

A. *Polymers*

Polymeric starting materials most suitable for the present networks are crosslinkable materials polymerized via peptide bonds, phosphate ester bonds and ether bonds. Exemplary polymers are natural product polymers derived from a living organism (i.e, of algal, microbial, animal, plant origin). Exemplary algal natural polymers include agar, furcelleran, alginate, carageenan. Exemplary plant natural polymers include starch, cellulose, pectin, gum arabic, guar gum, tracaganth, ghatti seed gums, locust bean gum. Exemplary microbial polymers include xanthan, pullalan, dextran, gellan. Exemplary animal polymers include chitin, chitosan, guar, heparin, hyaluronic acid and collagen.

Many of these are already KATP materials that include water-soluble, linear polymers such as polysaccharides (e.g. cellulose, food starch-modified (21 C.F.R. 172.892), chitin, chitosan, hyaluronic acid, xanthan gum (21 C.F.R. 172.695), chondroitin sulfate, heparin, and the like) and hydroxyalkyl, alkylhydroxyalkyl and alkyl-substituted cellulose derivatives such as cellulose

ethers. Exemplary cellulose ethers include methylcellulose (MC), hydroxyethylcellulose (HEC), ethylhydroxyethylcellulose (EHEC), hydroxyethylmethylcellulose (HEMC), hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), carboxymethylcellulose (CMC), and hydroxymethylcellulose (HMC). Cellulose ethers suitable for use in the present invention are readily available under a variety of trade names from a variety of manufacturers, for example: MC ("Methocel A"- Dow Chemical Company; "Metulose SM"- Shinetsu Chemical Company); HPMC ("Methocel E"- Dow Chemical Company; "Celacol HPM"- British Celanese Ltd.); HEC ("Cellosize WP"- Union Carbide Corporation); HPC (Aqualon Inc.).

Responsive polymers of amino acids such as poly(L-proline), and poly(valine-proline-glycine-X-glycine) [where X = tyrosine, phenylalanine, leucine, valine, glutamic acid, lysine, glycine, and other amino acids], as well as polypeptides like gelatin may also be used. Polynucleotides such as ribo- and deoxyribonucleotides are also suitable for crosslinking according to the invention.

Responsive polymers suitable for synthesis of KATP networks may also be KATP synthetic polymers such as polyethylene glycol (PEG)- e.g. 21 C.F.R. 172.210, 172.770, 172.820, 173.310; polyvinylalcohol (PVA- 21 C.F.R. 175.300 (xv)); polyethylene oxide (21 C.F.R. 172.770) and the like.

B. *Crosslinkers*

Crosslinkers are those chemical reagents with suitable KATP that are capable of linking polymer backbones. Specific crosslinkers will depend upon the polymer but preferred crosslinkers for polysaccharides, especially modified food starches and cellulose ethers, are multifunctional carboxylic acids, such as adipic acid (hexanedioic acid: $HOOC(CH_2)_4COOH$), succinic acid ($HOOC(CH_2)_2COOH$), malonic acid (propanedioic acid: $CH_2(COOH)_2$), sebacic acid (decanedioic acid: $HOOC(CH_2)COOH$), glutaric acid (pentanedioic acid: $HOOC(CH_2)_3COOH$), or any dicarboxylic acid (e.g. 1,10 decanedicarboxylic acid). Dicarboxylic hydroxyacids, such as tartaric acid and malic acid may also have suitable KATPs, as may multifunctional carboxylic acids such as 1,2,3,4-butanetetracarboxylic acid.

Other crosslinkers that have suitable KATPs include dialdehydes, such as glutaraldehyde, which are preferably utilized in an acidic environment. Irradiation energy is another useful crosslinker with a suitable KATP.

Persons having ordinary skill in the art will readily appreciate that esterification reactions between the hydroxyl groups of the ether and the carboxyl group on the preferred crosslinkers provides the crosslinkage arrangement. It will be further understood that certain ethers will participate in more active crosslinking than others. We have found that adipic acid will not produce a useful crosslinkage with HPC and HPMC while it will crosslink HEC. This is probably due to the stearic hindrance to esterification of the crosslinker afforded by the secondary and tertiary alcohols of HPC and HPMC, as opposed to the less sterically hindered primary alcohols of HEC.

Unsaturated dibasic acids have been used to physically crosslink water soluble polymers by application of drying and/or heat (see, for example, Reid U.S. Patent 3,379,720, incorporated herein by reference). Unfortunately, the heat required to crosslink water soluble polymers within a reasonable time of several hours is very high, ranging from 90° C (2-3 hour gelation) to 200° C (1-2 minute gelation). This may render the Reid method unsuitable for use with heat labile, biologically active compounds. At room temperature, the Reid methodology produced a gel in 10-30 days.

One of the present methods for crosslinking directly with multifunctional carboxylic acids involves azeotropic distillation to ensure that the water formed during the esterification reaction is continually removed from the reaction system. However, this method is limited to highly reactive compounds (see Examples).

We have, however, discovered that a preferred method of effective crosslinking may be accomplished in 3-4 hours by using acyl halide derivatives of multifunctional carboxylic acids as the crosslinker reagents added to the polymer solution. These acyl halides preferably are chloride derivatives such as adipoyl chloride, sebacoyl chloride, succinyl chloride, and the like. Acyl chloride derivatives of multifunctional carboxylic acids are very unstable in water and will react almost immediately to form the corresponding acid in solution (see, for

example, Pine et al., *Organic Chemistry*, supra, p. 319) and it is this acid, not its halide derivative, that becomes incorporated into the final form of the polymer network as the crosslinkage. As a result, one embodiment of the synthesis requires use of anhydrous conditions and anhydrous solvents but without the need for azeotropic distillation (see Examples). Furthermore, because the halide derivative is so reactive with water, aqueous leaching of a polymer network with any residual halide derivative will necessarily yield the acid form of the crosslinker in the leachate, not the halide derivative. Thus, the leachate is a KATP material.

C. *Functional Groups*

The three-dimensional polymer networks described herein may also be functionalized during synthesis with one or more surfactants, affinity ligands (e.g. monoclonal antibodies), chelators, enzymes, and the like that are immobilized in or on the polymer network. The term "immobilize" refers to physical trapping of a functionality within a polymer network as well as chemical bonding of a functionality via covalent, or other bonding, within a polymer network. Methods of immobilizing such functionalities to polymer networks are well known to the practitioner (see, for example, Hoffman et al. U.S. Patent 4,912,032; McCain et al. U.S. Patent 4,737,544; O'Driscoll et al. U.S. Patent 3,859,169, each of which is incorporated herein by reference).

For example, immobilization of a ligand within KATP polymer gel networks allows the ligand to be exposed to, and/or isolated from, an environment by changing the gel's volume (see discussion on "responsive" gels, below). That is, a substance may be delivered to or removed from an environment by employing a ligand immobilized within a responsive KATP polymer gel of the present invention. The immobilized ligand may be selected for its capability of specifically binding with a substance although the ligand may also bind non-specifically.

A ligand (e.g. an enzyme or antibody) may be physically trapped and protected within a polymer gel or may be immobilized to a polymer chain by

linkages that are labile to enzymes or aqueous solvent having a specific pH or temperature. A drug, antibody, or other molecule may be immobilized to, for example, cellulose ether moieties and the gel kept in a dry or shrunken state. When such a gel is swollen, it will incorporate solvent and if the solvent can degrade the labile linkage(s), the drug or antibody or other molecule will be released from the gel into the environment.

The material immobilized in the KATP polymer gels described herein may also be a binding component of an affinity binding pair. Suitable binding pairs include an antibody which binds with an antigen or hapten of interest; a receptor that binds with a hormone, vitamin, dye or lipid binding partner in solution; lectins that bind with polysaccharides; DNA or RNA that binds with complementary DNA,RNA, or oligonucleotides; ions that bind with chelators; and the like.

In another embodiment, a chemically active reactant may be immobilized to provide a means for controlling a reaction. Using the preferred responsive gels described below, reactions may be cycled on and off as exposure of the immobilized reactant to a reaction condition is regulated by altering the volumetric changes of the gel containing the reactant. Such a system could include an enzyme or antibody immobilized in or on a KATP gel of the present invention to catalyze a reaction with a substrate in a solution of interest (see, for example, Hoffman et al. U.S. Patent 4,912,032, incorporated herein by reference).

Most preferably, the functional moieties (e.g. affinity ligands) also have a known acceptable toxicological profile.

D. *Additives*

"Additives" are defined herein as materials incorporated into a responsive polymer gel network of the invention that have a KATP suitable for the particular use of that gel. Additives include, but are not limited to, stabilizers, biocides, anti-microbials, adhesives.

IV. Responsive Gel Networks

Preferred crosslinked polymer networks of the invention are gels that are "responsive"-- i.e. are gels that, when challenged with an environmental change, are affected by that environmental change in that the environmental change causes the entire gel, or a component thereof, to undergo a reversible volumetric change in which the gel expands from a less liquid-filled state or dry state to a more liquid-filled state; or collapses from a more liquid-filled state to a less liquid-filled state.

Polymer gels used in the present method may be expanded by either (i) contacting a dried gel with a solvent and allowing the gel to non-reversibly swell with solvent and incorporate any moiety (e.g. a drug, affinity ligand) contained in the solvent; (ii) initiating a reversible volumetric expansion of the gel to incorporate solvent (and any moiety contained therein) by triggering the expansion with a stimulus; or (iii) a combination of (i) and (ii). In this context, the term "incorporate" refers to both absorption of a material inside the gel network and adsorption of a material on a surface of the gel.

The degree of volumetric change between collapsed and expanded states of the preferred "responsive" gels at their particular environmental transition region is quantitatively much greater than the volume change of the gel outside the environmental transition region. Equations describing this volume behavior are not simple monotonic functions (as they are for conventional hydrogels) but contain one particular environmental transition where the volume change is much larger than at other environmental transitions for the same gel.

The primary requirement of a responsive polymer gel of the present invention is that the entire gel, or a component, undergo a volume change in response to a change in environmental condition. The gel as a whole may meet these requirements. Nevertheless, the gel may itself include several other (i.e. non-responsive) components as long as at least one component(s) provides the required property.

For instance, the gel may be a single material such as a single polymer network which meets the volumetric response requirement. The gel may also be a co-polymer, whether a random, alternating, or block co-polymer, that has a KATP

and which meets the volumetric response requirement. The gel may also include two or more polymers, each component polymer having a KATP, so long as the result is a physical polymer blend, wherein at least one polymer meets the volumetric response requirement.

The gel may also be an interpenetrating polymer network (IPN) in which each KATP polymer maintains its properties. An IPN may possess only a volume change property such as an IPN of HPC and carboxymethylcellulose. A responsive gel may also be combined in an IPN with a sorptive-type gel to meet the requirements of vapor extraction, drug delivery, electrophoresis, or other delivery system. Thus, a purely responsive polymer may itself be combined in an IPN with a polymer that has a sorptive component. The IPN may possess both properties, however, so that at least one polymer member of the IPN provides the sorptive property and at least another polymer member provides the volume change property. This type of configuration is particularly useful in drug delivery systems.

The reversible volume change of the entire gel, or a component thereof, may be either continuous or discontinuous. A "continuous" volume change is marked by a reversible change in volume (i.e. a collapse or swelling) that occurs over a relatively large change in environmental condition. Moreover, there exists at least one stable volume near the transition between the swollen and collapsed states.

Crosslinked gels of the invention may undergo a "discontinuous" volume change in which the reversible transition from swollen to collapsed states, and back again, occurs over an extremely small change in environmental condition, such as less than 0.1 °C or 0.1 pH unit. Such reversible gels are often called "phase-transition" gels (see, for example, Hirotsu et al., J. Chem. Phys. 87:15, 1987, which describes synthetic polymeric gels that undergo phase transitions). There is no stable volume between the swollen and collapsed states at the phase-transition and, in theory, the expansion and/or collapse occurs over an infinitesimally small environmental change. A gel undergoing a continuous phase-

transition may have a similar order of magnitude total volume change as a gel undergoing a discontinuous phase-transition.

On a molecular level, the preferred responsive gels are sensitive to small changes in a restricted repertoire of environmental "trigger" conditions consisting primarily of temperature, pH, solvent concentration, and ion concentration. On a macroscopic level, any of a variety of environmental changes may be imposed on the gel which allows the specific trigger to induce a volume change. These environmental conditions may be, but are not necessarily, the same as the trigger and include, but are not limited to, a change in temperature, electric field (presence, strength, and/or orientation), magnetic field (presence, strength, an/or orientation), photon energy, pH, solvent composition, ion concentration, concentration of biomolecules, mechanical force, pressure, and the like.

The preferred gels of the invention may be combined with a material that acts as a molecular "transducer", converting an environmental condition into an appropriate trigger. For example, a dye may be introduced into a temperature-triggered responsive gel. The dye is designed to trap light of a given wavelength and convert the light energy into heat, thus triggering the gel to undergo a temperature-induced volume change (see, for example, Seasick et al. Nature 346:6282, 1990, incorporated herein by reference).

The volumetric changes of gels described herein result from competition between intermolecular forces, usually electrostatic in nature, that act to expand the polymer network; and at least one attractive force that acts to shrink it.

Volumetric changes in aqueous gels are driven primarily by four fundamental forces: ionic, hydrophobic, hydrogen bonding and van der Waals bonding interactions, either alone or in combination. Each of these interactions may be independently responsible for a volume transition in preferred gels of the invention. Each of these fundamental forces is most strongly affected by a particular trigger. Changes in solvent concentration most strongly affect the van der Waals interaction; changes in temperature most strongly affect hydrophobic interactions and hydrogen bonding; and changes in pH and ion concentration most strongly affect ionic interactions.

Thus, a gel whose volume change is governed by ionic interactions would include components that are weakly acidic and weakly basic, such as poly(methylmethacrylate)/dimethylaminoethyl methacrylate (see, for example, Siegel et al. Macromolecules 21:3254, 1988) and cellulose ethers such as HPC crosslinked by methods described herein. Gels of this type are sensitive to pH (see Example 1).

Gels whose volume change is governed by hydrogen bonding will collapse with a decrease in temperature and are exemplified by interpenetrating polymers that comprise acrylamide as one polymer, acrylic acid as the other polymer, and water as the liquid medium. Gels whose volume change is governed by hydrophobic interactions will collapse when challenged with an increase in temperature and are exemplified by N-isopropylacrylamide. Gels whose volume change is governed by van der Waals interactions will behave similarly to those governed by hydrophobic interactions.

Gels may be formulated in which the volume change is governed by more than one fundamental force. In particular, gels consisting of copolymers of positively and negatively charged groups meet this requirement. In these gels, polymer segments interact with each other through ionic interactions and hydrogen bonding. The combination of these forces results in the existence of several pH-driven phases (see, for example, Annaka et al. Nature 355:430, 1992, incorporated herein by reference).

Volumetric changes in gels can be derived from their equations of state that relate three characteristic state variables of the gel: volume (V) or equivalent density of the polymer network (ϕ), temperature (T) plus polymer-solvent interaction parameter (ΔF), and the osmotic pressure(π). At equilibrium, the osmotic pressure of a gel must be zero ($\pi=0$).

Without wishing to be bound by any particular theory, and as but one example of the theories developed in the field, one may determine the temperature (Tc) of the phase transition where (Θ) is the theta temperature of the polymer network in the solvent, and ϕ_o is the concentration of the polymer network when in a random walk configuration, using equation 1.

(Equation 1) $T_c = \Theta/(1 \pm 22.5\phi_o)$

The value in the denominator is positive for gels that collapse at lower temperature (see Example 4) and negative for gels that collapse at higher temperatures (see Examples 1 and 2).

Three osmotic pressures contribute to the total osmotic pressure of a gel, as shown below in equations 2, 3, 4 and 5.

(Equation 2) $\pi = \pi_{rubber} + \pi_{affinity} + \pi_{ion}$

(Equation 3) $\pi_{rubber} = v_o kT\{(\phi/2\phi_o) - (\phi/\phi_o)^{1/3}\}$

(Equation 4) $\pi_{affinity} = v_o kT\{\ln(1 - \phi) + \phi\} + \Delta F\ (\phi/\phi_o)^2$

(Equation 5) $\pi_{ion} = v_o kT\{(\phi/\phi_o)\}$

Here, V_o denotes the number of effective crosslinks of the network when it is in the random walk configuration whose density is denoted by ϕ_o. This state is referred to as the reference state. The rubber elasticity, π_{rubber}, which originates from the configurational entropy of the polymer network, provides a restoring pressure back to the reference polymer network density. When a polymer network is expanded, a negative pressure is created in the network and shrinks back. On the other hand, when it is contracted, the pressure acts to expand to the original reference state. Secondly, the polymer-polymer and polymer-solvent interactions give rise to another osmotic pressure, $\pi_{affinity}$. In a poor solvent, the polymer network tends to shrink, whereas in a good solvent a gel tends to swell. The last factor is the osmotic pressure due to ionization of the polymer network, π_{ion}. The counter-ions within the gel create a gas-type pressure to expand the gel in proportion to the density of counter-ions as well as the absolute temperature, kT, where k is the Boltzmann constant.

These three osmotic pressures compete with each other and the gel volume is equilibrated in a condition at which these three osmotic pressures balance at $\pi = 0$. There is a special condition at which the competing pressures become equal to each other, at which point the volume change occurs. When the ionization pressure is large, as in the case of extensively ionized gels, the volume change is physically dramatic and discontinuous. With increased ionization, the volume change becomes large. There exists a minimum critical concentration of ionic component within a gel

sorbent for each solvent system employed in order to achieve reversible discontinuous volume change of the gel sorbent. This minimum ionic concentration can be determined for each polymer network and solvent system. For some polymer systems, no ionization is required.

The equations above qualitatively explain all of these aspects of volumetric changes (see, for example, Tanaka et al. Phys. Rev. Lett. 45:1636, 1980; Tanaka et al. U.S. Patent 5,100,933; each of which is incorporated herein by reference; see also Gehrke Adv. Polymer Sci. 110:81, 1993 for other theoretical descriptions).

Persons having ordinary skill in the art may readily test whether any particular polymer network is responsive by following the procedures, and using the apparatus described in Example 1 to measure the volumetric change of the network as a function of, for instance, temperature, solvent concentration, pH and the like.

V. Methods of Preparing Safe Polymer Gel Networks

A. *General Considerations*

Any of a variety of techniques may be used to make the safe polymer gel networks of the present invention.

A general protocol for forming a KATP polymer network of the present invention using a crosslinkable polymer includes the steps of dissolving the KATP polymer(s) in a suitable solvent and allowing the polymer(s) and solvent to mix. A crosslinking agent is then added to the polymer solution, and the solution and crosslinker are further mixed together. The resulting solution may be poured into a solid mold (e.g. between two glass plates), and the crosslinking reaction carried out.

In an exemplary sequence, a chemical crosslinking reaction is carried out in the homogenous polymer state at room temperature to form a certain amount of polymer network. Total crosslinking time will vary but is generally less than 24 hours. The network is then removed from its mold, and repeatedly washed to leach out any leachable material present in the network. In principle, a polymer network can be made from any KATP polymer with side groups that can react with a di- or multi-functional crosslinking molecule.

The polymer solution may also be formed into beads or spheres using crosslinking in a non-solid mold where the reacting solution (polymer, crosslinker and catalyst, if needed) is dispersed in a non-solvent to form a droplet. The solution reacts within the droplet to form a bead. In this method, the non-solvent may be considered to be a "mold" for polymer network droplets.

U.S. Patent No. 3,208,994 to Flodin et al., incorporated herein by reference, generally discloses methods of preparing polysaccharide gel beads using suspension crosslinking. One introduces a water soluble polysaccharide and crosslinker into a suspension medium under agitation to obtain suspended drops of the polysaccharide solution. Another method of preparing gel beads uses inverse emulsion polymerization, in which a monomer solution is introduced into a dispersion solvent to form monomer droplets and polymerization is initiated to form polymer gel beads (see, for example, Hirose et al. Macromolecules 20:1342, 1987, incorporated herein by reference). Preferably, an aqueous cellulose ether solution, a non-polar saturated hydrocarbon solvent, and a crosslinker are provided and admixed to form a two-phase system. The two-phase system is agitated sufficiently to form droplets of aqueous cellulose ether solution in the two-phase system. The agitation of the two-phase system is maintained to form crosslinked cellulose ether gel beads and the crosslinked cellulose ether gel beads are thereafter recovered from the two-phase system.

Polymer networks of the invention also may consist, in whole or in part, of polymers made by copolymerization/crosslinking of monofunctional and polyfunctional polymerizable monomers.

A preferred method for making KATP gels from cellulose ethers involves dissolving a sample of cellulose ether such as HPC or HPMC in an anhydrous solvent that does not contain active hydrogen, such as for example N-methyl pyrolidone (21 C.F.R. 176.300), dimethylsulfoxide (DMSO), dimethylformamide (DMF), methylethlketone (MEK), tetrahydrofuran (THF), and the like. The concentration of polymer in the solution may range from about 5-20% by weight of polymer per volume of solution, with a preferred concentration primarily a function of the kind of polymer used in the synthesis. The molecular weight of the cellulose ether should be at least about 20,000. Preferred molecular weights range from about 75,000 to about

150,000. The higher the molecular weight of the polymer, the sharper will be the volume change of the resulting responsive gel. This is because a higher molecular weight will result in formation of a more consistent three-dimensional polymer network. Molecular weights may range up to 1,000,000 or more although it will be understood that viscosity effects will place an upper limit on the molecular weight of the polymer starting material. Those having ordinary skill in the art may readily determine using the methods described herein the extent to which viscosity constraints interfere with the gel formation process and/or prevent the crosslinker from mixing with the polymer.

When synthesizing gels of the invention with a crosslinker reagent that is a dicarboxylic acid, azeotropic distillation is a preferred method. A first solvent such as DMSO is added to a distillation flask containing the polymer and crosslinker reagents. Both are mixed to achieve a clear solution. To this solution, a small amount (several hundred μL) of an acidified solution of first solvent is added, followed by the addition of a second solvent (e.g. toluene). This solution is allowed to react under azeotropic distillation until a gel forms in the flask. The gel is then removed and placed in an excess of deionized water. The water is removed and excess primary alcohol (e.g. methanol) is added to remove excess solvent. The gel is washed and then dried in a desiccator.

Synthesis of KATP gels using acyl halide derivatives of dicarboxylic acids generally occurs as follows: While stirring the cellulose ether polymer solution under anhydrous conditions, the solution is cooled slightly below room temperature (in some embodiments to between about 10-20 °C) and a cold solution (in some embodiments between about 2-8 °C) of a preferred acyl halide derivative of a multifunctional carboxylic acid is added as crosslinker to the polymer solution. This solution is stirred and then allowed to sit until gelation has occurred. Gelation time will necessarily vary and may occur within about 2 hours (e.g. for HPC) or as long as 24 hours (e.g. for HPMC). The polymer/crosslinker weight ratio is between about 12/1 and 8/1. The lower the ratio, the more highly crosslinked the resulting gel will be.

The reaction will not always produce polymer/crosslinker/polymer covalent couplings, and a number of incomplete crosslinks will occur that will leave one end of

the acid chloride group unreacted. After the crosslinking reaction, any unreacted acid chloride is quantitatively reacted with water to produce a carboxylic acid group. Carboxylic acid groups in the polymer network will provide a pH- and temperature-responsive gel (see Example 1).

Alternatively, if it is desired to produce a gel which has basic (amine) groups rather than acid groups, this may be achieved for example by allowing the acyl halide, cellulose ether reaction product to react with a KATP diamine such as ethylenediamine or hexamethylenediamine (21 C.F.R. 175.300 (b) (3) (xxxii) to produce an amine-terminated amide. The amine-terminated amide will survive the workup. These amine groups will cause the gel to be pH and temperature responsive in a range different from the acid group-containing gel.

After the gel is formed, destruction of any remaining acid chloride groups is carried out by soaking the gel in distilled water for about 12 hours. Solvent is then removed by soaking the gel in an alcohol (e.g. methanol, ethanol, and the like) for at least several hours so that the methanol can diffuse into the gel and the solvent can diffuse out of the gel. After several hours, the wash is drained off. This process is repeated at least 4-5 times. The gel is then washed 4-5 times with distilled water while it is being heated to between about 60-80 °C for about 2 hours. Heating drives off any remaining alcohol, leaving gel and water. The process is repeated at least 3-5 times until the gel appears opaque at the elevated temperature. This opacity signifies that the gel has undergone a volumetric change at a lower critical solution temperature (LCST), and therefore that the gel has temperature responsive characteristics. For HPC, the LCST is between 42 and 46 °C. The degree of responsiveness to pH may be assayed using the device and procedures given in Example 1. In the Examples, all the gels were pH responsive, and all gels except those of Examples 3, 6-11 were also temperature responsive.

Persons having ordinary skill in the art may readily determine if a particular KATP polymer material is capable of forming a polymer gel network by following the synthesis procedures described herein. Moreover, methods to determine the degree of crosslinking are conventional and are described, for example, by Peppas et al. (eds) in "Hydrogels in Medicine and Pharmacy" Vol. 1, CRC Press, Boca Raton, Florida,

1986. The degree of crosslinking of polymer gels may also be measured by uniaxial compression tests. Briefly, a cylindrical gel disk (approximately 25 mm in diameter) is first swollen to equilibrium in water at 25°C and weight, thickness, and diameter measured using a balance, micrometer, and a ruler, respectively. The gel sample is placed in a water-filled Petri dish and a constant strain applied by adjustment of a micrometer. The relaxation of the applied stress was monitored by computer until the equilibrium, relaxed state was reached. Then the strain is increased in steps and equilibrium value of stress at each point recorded. Next, the equilibrium stress is plotted versus the strain function ($\alpha - \alpha^{-2}$), where a is the ratio of deformed thickness to the unstrained thickness of the sample. This plot is expected to be linear for $\alpha > 0.90$. The shear modulus is obtained from the slope of the initial linear region of the plot using the equations of Mark (see Physical Properties of Polymers Am. Chem. Soc. Wash. D.C., 1984, incorporated herein by reference). The crosslink density of the gel sample is calculated from the equations derived by Harsh et al. (see J. Control Release, 1991, incorporated herein by reference) for non-porous gels, e.g.

$$G = RT\ \rho x\ (\phi 2f/\phi 2)^{2/3}$$

where:

ρx is the crosslink density;

$\phi 2f$ is the polymer volume fraction at the network formation; and

$\phi 2$ is the polymer volume fraction of the gel during the experiment.

B. *Microporous Gels*

Microporous KATP gels are encompassed by the invention. A gas phase is dispersed throughout a fluid polymer phase and the resulting porous material is solidified. In such microporous materials, the cell or pore size is generally of the order of 100-200 microns or larger (see, for example, Aubert et al. Macromolecule, 21:3468, 1988, incorporated herein by reference).

Another method for fabricating microporous gels is to disperse solid particles in a polymer melt or in a polymer solution. The polymer solution or melt is solidified either by chemical crosslinking or by physical means such as freezing. After

solidification of the polymer, the solid particles are leached away (see, for example, Mikos et al. Mater. Res. Soc. Symp. Proc. 252:353, 1992, incorporated herein by reference).

Microporous gels may also be formed by a process in which co-monomers including crosslinker are polymerized in one phase of a bicontinuous microemulsion, while the other phase forms the cells or pores (see, for example, Hainey et al. Macromolecules 24:117, 1991, incorporated herein by reference). Materials made by this process have a pore size ranging from 1-30 microns. This technique is limited by the ability to find a suitable solvent and non-solvent for the comonomers and emulsifying agent which will form a bicontinuous emulsion.

A preferred process for making microporous gels of the present invention, and for developing design controls that regulate the microstructure of the final product, is the phase inversion process. "Phase inversion", hereinafter called "phase separation", refers to the process by which a polymer solution containing one or more polymer precursors, in which the solvent is the continuous phase, inverts into a three-dimensional network or gel where the polymer(s) are now the continuous phase. Phase separation occurs when polymer becomes insoluble in the solvent upon changing the system conditions (see, for example, Kesting *Synthetic Polymeric Membranes: A Structural Perspective*, J. Wiley and Sons, NY, 1985, incorporated herein by reference). Thus, one method of the invention includes contacting a dissolved polymer with another solvent that effectively removes the solvent from the polymer and precipitates the polymer out of solution, forming a microporous interconnected structure that is crosslinked to convert it into a responsive gel.

Most preferred are methods in which temperature induces phase separation. These processes use a substance that is a good solvent for the polymer at one temperature, but is a poor solvent at another temperature. Temperature may be easily controlled so that this method generally is reproducible, since heat transfer is much faster than mass transfer.

A preferred method described herein for making microporous volume change gels can be applied to make crosslinked microporous gels from any crosslinkable polymer-solvent system which phase separates with changes in temperature. Many

aqueous-soluble polymers phase separate with changes in temperature. Even aqueous polymer solutions which don't phase separate at a particular temperature can be forced to phase separate at another temperature by adding, for example, an organic solvent such as ethanol or a suitable salt, such as, for example, 1 M NaCl.

Microporous gels may be "fast response" gels. As defined herein, "fast response" means that the gel reaches 90% of its maximum volumetric swelling or 90% of its minimum volumetric collapse in a time that is at least ten times faster than a comparable non-porous gel of the same geometry when both gels are subjected to a similar change in an environmental condition. Methods of making and using fast response gels may be found in co-pending PCT application number PCT/US94/05400, filed 13 May 1994 (35 U.S.C. Section 371(c) (2): "Microporous Fast Response Gels and Methods of Use"- Gehrke and Kabra).

VI. Loading Biologically Active Compounds into Polymer Gel Networks

It will be understood that there are many methods available to load biologically active compounds into a polymer gel. Two general methods of drug loading into gels have been used: (i) formation of a hydrogel in the presence of the compound (i.e. drug); and (ii) swelling of a preformed gel in a solution (i.e. an organic solvent) of the compound (i.e. drug; see, for example, Kim et al. Phar. Res. 9:283, 1992). Loadings are generally on the order of about 3 percent by weight.

Gref et al. (Science 263:1600, 1994) have developed biodegradable nanospheres using amphiphilic polymers that phase-separated during emulsification. Up to 45 percent by weight of drug loading was achieved by dissolving the drug in the same organic solvent that dissolved the copolymer. Although drug loading is high using this method, the drug must be dissolved in an organic solvent.

Recently, principles for isolating and purifying proteins from solution by sorption into a crosslinked polymer gel phase have been derived, the sorption driven by addition of a substantially immiscible, second polymer phase to the protein solution (see, for example, Gehrke et al. Biotechnol. Progr. 7:355, 1991, incorporated herein by reference). This method, also called "solution-controlled gel sorption", is based in part on the discovery that the general principles of two-phase aqueous extraction used

for purifying proteins can be extended to be used in which one of the phases is a crosslinked gel. In two-phase aqueous extraction methods of protein purification, a protein is made to partition selectively into one of two immiscible aqueous polymer solution phases which are in contact with each other.

In particular, high loadings of biologically active compounds into gels may be obtained with solution controlled gel sorption using a second, loading polymer phase. In the presence of a salt solution, the second loading polymer and salt have a synergistic effect which causes partitioning and sorption (exceeding 20 % by weight) of the compound into the polymer gel. The second loading polymer need not be a gel but is most preferably soluble in the same solvent that is the gel's solvent.

Solution-controlled gel sorption is utilized in the Examples presented herein for loading a solute into a crosslinked gel. The partitioning behavior is governed by properties such as molecular weight of the polymers, the type and concentration of salts and the relative hydrophobicity/hydrophilicity of the solute. Differences in the various interaction energies between the solute and the different polymers leads to a partition coefficient (concentration of solute in the gel/concentration of the solute in the second loading polymer) greater than one (i.e. preferential loading by the gel) or less than one (i.e. preferential loading by the second loading polymer).

The general method used for solute loading into a crosslinked gel phase is briefly as follows:

Crosslinked gels are pre-equilibrated with solute-free, loading polymer solutions. The equilibrated gels are then separated from the loading polymer solution. To each gel, a solution with the same loading polymer concentration as the pre-swelling solution but including a solute and a salt is added. The tube is then agitated to mix gel and salt/solute solution. Equilibrium is reached in less than 15 minutes (see Gehrke et al., 1991, supra), and the gel separated from the remaining solution. The solute concentration in the second, loading polymer phase may be determined by a variety of methods, depending upon the solute of interest. For spectrophotometric assays, light absorbance is measured at 280 nm for proteins; at 630 nm for blue dextran; and at 520 nm for Vitamin B12 with a UV/VIS spectrophotometer. The concentration of solute in the gel phase is determined by a mass balance.

Cellulose ethers are advantageous for loading by this method. Because the degree of substitution of the anhydroglucose unit has a great effect on the degree of hydrophilicity, cellulose ethers differ in their hydrophilic nature. There are a number of immiscible polymer gels that are mainly water and that are close to each other on a spectrum of relative hydrophobicity-hydrophilicity. This means that phase systems formed by these polymers, including cellulose ethers, can be expected to be selective in separating substances which themselves are mainly water; that is substances that fall within the same part of the solvent spectrum. Examples are particles and macromolecules of biological origin. Aqueous solutions of the following polymers are mutually immiscible and are ranked in order of increasing hydrophobicity: dextran sulfate, carboxymethyl dextran, dextran, hydroxypropyldextran, methylcellulose, hydroxypropylcellulose, polyvinylalcohol, polyethylene glycol and polypropylene glycol.

Solute is recovered from the loaded polymer as follows: Solute is chosen to have a very low partition coefficient in pure buffer, lacking any polymer. The solute contained in the loaded gel after the partitioning experiment is recovered by adding pure buffer lacking any to the loaded gel. The gel is separated by centrifugation from any supernatant and the concentration of the solute in the supernatant measured using a spectrophotometer. This procedure is repeated until the solute concentration in the supernatant is negligible.

With a reversibly responsive gel, solute recovery can be accomplished by causing the gel to undergo to volumetric collapse using established methods (see, for example, Cussler U.S. Patent 4,555,344, incorporated herein by reference).

VII. Biologically Active Compounds

The biologically active compounds that may be loaded into the polymer networks of the present invention are any substance having biological activity, including proteins, polypeptides, polynucleotides, nucleoproteins, polysaccharides, glycoproteins, lipoproteins, and synthetic and biologically engineered analogs thereof.

Examples of biologically active compounds that might be utilized in a delivery application of the invention include literally any hydrophilic or hydrophobic

biologically active compound. Preferably, though not necessarily, the drug is one that has already been deemed safe and effective for use by the appropriate governmental agency or body. For example, drugs for human use listed by the FDA under 21 C.F.R. 330.5, 331 through 361; 440-460; drugs for veterinary use listed by the FDA under 21 C.F.R. 500-582, incorporated herein by reference, are all considered acceptable for use in the present novel polymer networks.

Drugs that are not themselves liquid at body temperature can be incorporated into polymers, particularly gels. Moreover, peptides and proteins which may normally be lysed by tissue-activated enzymes such as peptidases, can be passively protected in gels as well.

The term "biologically active compound" includes pharmacologically active substances that produce a local or systemic effect in animals, plants, or viruses. The term thus means any substance intended for use in the diagnosis, cure, mitigation, treatment or prevention of disease or in the enhancement of desirable physical or mental development and conditions in an animal, plant, or virus. The term "animal" used herein is taken to mean mammals, such as primates, including humans, sheep, horses, cattle, pigs, dogs, cats, rats, mice; birds; reptiles; fish; insects; arachnids; protists (e.g. protozoa); and prokaryotic bacteria. The term "plant" means higher plants (angiosperms, gymnosperms), fungi, and prokaryotic blue-green "algae" (i.e. cyanobacteria).

The biologically active compound may be any substance having biological activity, including proteins, polypeptides, polynucleotides, nucleoproteins, polysaccharides, glycoproteins, lipoproteins, and synthetic and biologically engineered analogs thereof. The term "protein" is art-recognized and for purposes of this invention also encompasses peptides. The proteins or peptides may be any biologically active protein or peptide, naturally occurring or synthetic.

Examples of proteins include antibodies, enzymes, steroids, growth hormone and growth hormone-releasing hormone, gonadotropin-releasing hormone, and its agonist and antagonist analogues, somatostatin and its analogues, gonadotropins such as luteinizing hormone and follicle-stimulating hormone, peptide-T, thyrocalcitonin, parathyroid hormone, glucagon, vasopressin, oxytocin, angiotensin I and II,

bradykinin, kallidin, adrenocorticotropic hormone, thyroid stimulating hormone, insulin, glucagon and the numerous analogues and congeners of the foregoing molecules.

Classes of biologically active compounds which can be loaded into crosslinked gels using the methods of this invention include, but are not limited to, anti-AIDS substances, anti-cancer substances, antibiotics, immunosuppressants (e.g. cyclosporine) anti-viral substances, enzyme inhibitors, neurotoxins, opioids, hypnotics, antihistamines, lubricants tranquilizers, anti-convulsants, muscle relaxants and anti-Parkinson substances, anti-spasmodics and muscle contractants, miotics and anti-cholinergics, anti-glaucoma compounds, anti-parasite and/or anti-protozoal compounds, anti-hypertensives, analgesics, anti-pyretics and anti-inflammatory agents such as NSAIDs, local anesthetics, ophthalmics, prostaglandins, anti-depressants, anti-psychotic substances, anti-emetics, imaging agents, specific targeting agents, neurotransmitters, proteins, cell response modifiers, and vaccines.

A more complete listing of classes of compounds suitable for loading into polymers using the present methods may be found in the *Pharmazeutische Wirkstoffe* (Von Kleemann et al. (eds) Stuttgart/New York, 1987, incorporated herein by reference). Examples of particular biologically active substances are presented below:

<u>Anti-AIDS substances</u> are substances used to treat or prevent Autoimmune Deficiency Syndrome (AIDS). Examples of such substances include CD4, 3'-azido-3'-deoxythymidine (AZT), 9-(2-hydroxyethoxymethyl)-guanine acyclovir(), phosphonoformic acid, 1-adamantanamine, peptide T, and 2',3' dideoxycytidine.

<u>Anti-cancer substances</u> are substances used to treat or prevent cancer. Examples of such substances include methotrexate, cisplatin, prednisone, hydroxyprogesterone, medroxyprogesterone acetate, megestrol acetate, diethylstilbestrol, testosterone propionate, fluoxymesterone, vinblastine, vincristine, vindesine, daunorubicin, doxorubicin, hydroxyurea, procarbazine, aminoglutethimide, mechlorethamine, cyclophosphamide, melphalan, uracil mustard, chlorambucil, busulfan, carmustine, lomusline, dacarbazine (DTIC: dimethyltriazenomidazolecarboxamide), methotrexate,

fluorouracil, 5-fluorouracil, cytarabine, cytosine arabinoxide, mercaptopurine, 6-mercaptopurine, thioguanine.

Antibiotics are art recognized and are substances which inhibit the growth of or kill microorganisms. Antibiotics can be produced synthetically or by microorganisms. Examples of antibiotics include penicillin, tetracycline, chloramphenicol, minocycline, doxycycline, vanomycin, bacitracin, kanamycin, neomycin, gentamycin, erythromicin and cephalosporins.

Anti-viral agents are substances capable of destroying or suppressing the replication of viruses. Examples of anti-viral agents include a-methyl-P-adamantane methylamine, 1,-D-ribofuranosyl-1,2,4-triazole-3 carboxamide, 9-[2-hydroxy-ethoxy]methylguanine, adamantanamine, 5-iodo-2'-deoxyuridine, trifluorothymidine, interferon, and adenine arabinoside.

Enzyme inhibitors are substances which inhibit an enzymatic reaction. Examples of enzyme inhibitors include edrophonium chloride, N-methylphysostigmine, neostigmine bromide, physostigmine sulfate, tacrine HCL, tacrine,1-hydroxy maleate, iodotubercidin, p-bromotetramisole, 10-(alpha-diethylaminopropionyl)- phenothiazine hydrochloride, calmidazolium chloride, hemicholinium-3, 3,5-dinitrocatechol, diacylglycerol kinase inhibitor I, diacylglycerol kinase inhibitor II, 3-phenylpropargylamine, N^6-monomethyl-L-arginine acetate, carbidopa, 3-hydroxybenzylhydrazine HCl, hydralazine HCl, clorgyline HCl, deprenyl HCl,L(-)-, deprenyl HCl,D(+)-, hydroxylamine HCl, iproniazid phosphate, 6-MeO-tetrahydro-9H-pyrido-indole, nialamide, pargyline HCl, quinacrine HCl, semicarbazide HCl, tranylcypromine HCl, N,N-diethylaminoethyl-2,2-diphenylvalerate hydrochloride, 3-isobutyl-1-methylxanthne, papaverine HCl, indomethacind, 2-cyclooctyl-2-hydroxyethylamine hydrochloride, 2,3-dichloro-a-methylbenzylamine (DCMB), 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine hydrochloride, p-aminoglutethimide, p-aminoglutethimide tartrate,R(+)-, p-aminoglutethimide tartrate,S(-)-, 3-iodotyrosine, alpha-methyltyrosine,L-, alpha -methyltyrosine,D L-,

acetazolamide, dichlorphenamide, 6-hydroxy-2-benzothiazolesulfonamide, and allopurinol.

Neurotoxins are substances which have a toxic effect on the nervous system, e.g. nerve cells. Neurotoxins include adrenergic neurotoxins, cholinergic neurotoxins, dopaminergic neurotoxins, and other neurotoxins. Examples of adrenergic neurotoxins include N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride. Examples of cholinergic neurotoxins include acetylethylcholine mustard hydrochloride. Examples of dopaminergic neurotoxins include 6-hydroxydopamine HBr, 1-methyl-4-(2-methylphenyl)-1,2,3,6- tetrahydro-pyridine hydrochloride, 1-methyl-4-phenyl-2,3- dihydropyridinium perchlorate, N-methyl-4-phenyl-1,2,5,6-tetrahydropyridine HCl, 1-methyl-4-phenylpyridinium iodide.

Opioids are substances having opiate like effects that are not derived from opium. Opioids include opioid agonists and opioid antagonists. Opioid agonists include codeine sulfate, fentanyl citrate, hydrocodone bitartrate, loperamide HCl, morphine sulfate, noscapine, norcodeine, normorphine, thebaine. Opioid antagonists include nor-binaltorphimine HCl, buprenorphine, chlornaltrexamine 2HCl, funaltrexamione HCl, nalbuphine HCl, nalorphine HCl, naloxone HCl, naloxonazine, naltrexone HCl, and naltrindole HCl.

Hypnotics are substances which produce a hypnotic effect. Hypnotics include pentobarbital sodium, phenobarbital, secobarbital, thiopental and mixtures, thereof, heterocyclic hypnotics, dioxopiperidines, glutarimides, diethyl isovaleramide, a-bromoisovaleryl urea, urethanes and disulfanes.

Antihistamines are substances which competitively inhibit the effects of histamines. Examples include pyrilamine, chlorpheniramine, tetrahydrazoline, and the like.

Lubricants are substances that increase the lubricity of the environment into which they are delivered. Examples of biologically active lubricants include water and saline.

Tranquilizers are substances which provide a tranquilizing effect. Examples of tranquilizers include chloropromazine, promazine, fluphenzaine, reserpine, deserpidine, and meprobamate.

Anti-convulsants are substances which have an effect of preventing, reducing, or eliminating convulsions. Examples of such agents include primidone, phenytoin, valproate, Chk and ethosuximide.

Muscle relaxants and anti-Parkinson agents are agents which relax muscles or reduce or eliminate symptoms associated with Parkinson's disease. Examples of such agents include mephenesin, methocarbomal, cyclobenzaprine hydrochloride, trihexylphenidyl hydrochloride, levodopa/carbidopa, and biperiden.

Anti-spasmodics and muscle contractants are substances capable of preventing or relieving muscle spasms or contractions. Examples of such agents include atropine, scopolamine, oxyphenonium, and papaverine.

Miotics and anti-cholinergics are compounds which cause bronchodilation. Examples include echothiophate, pilocarpine, physostigmine salicylate, diisopropylfluorophosphate, epinephrine, neostigmine, carbachol, methacholine, bethanechol, and the like.

Anti-glaucoma compounds include betaxalol, pilocarpine, timolol, timolol salts, and combinations of timolol, and/or its salts, with pilocarpine.

Anti-parasitic, -protozoal and -fungals include ivermectin, pyrimethamine, trisulfapyrimidine, clindamycin, amphotericin B, nystatin, flucytosine, natamycin, and miconazole.

Anti-hypertensives are substances capable of counteracting high blood pressure. Examples of such substances include alpha-methyldopa and the pivaloyloxyethyl ester of alpha-methyldopa.

Analgesics are substances capable of preventing, reducing, or relieving pain. Examples of analgesics include morphine sulfate, codeine sulfate, meperidine, and nalorphine.

Anti-pyretics are substances capable of relieving or reducing fever and anti-inflammatory agents are substances capable of counteracting or suppressing inflammation. Examples of such agents include aspirin (salicylic acid), indomethacin, sodium indomethacin trihydrate, salicylamide, naproxen, colchicine, fenoprofen, sulindac, diflunisal, diclofenac, indoprofen and sodium salicylamide.

Local anesthetics are substances which have an anesthetic effect in a localized region. Examples of such anesthetics include procaine, lidocain, tetracaine and dibucaine.

Ophthalmics include diagnostic agents such as sodium fluorescein, rose bengal, methacholine, adrenaline, cocaine, and atropine. Ophthalmic surgical additives include alpha-chymotrypsin and hyaluronidase.

Prostaglandins are art recognized and are a class of naturally occurring chemically related, long-chain hydroxy fatty acids that have a variety of biological effects.

Anti-depressants are substances capable of preventing or relieving depression. Examples of anti-depressants include imipramine, amitriptyline, nortriptyline, protriptyline, desipramine, amoxapine, doxepin, maprotiline, tranylcypromine, phenelzine, and isocarboxazide.

Anti-psychotic substances are substances which modify psychotic behavior. Examples of such agents include phenothiazines, butyrophenones and thioxanthenes.

Anti-emetics are substances which prevent or alleviate nausea or vomiting. An example of such a substance includes dramamine.

Imaging agents are agents capable of imaging a desired site, e.g. tumor, *in vivo*. Examples of imaging agents include substances having a label which is detectable *in vivo*, e.g. antibodies attached to fluorescent labels. The term antibody includes whole antibodies or fragments thereof.

Specific targeting agents include agents capable of delivering a therapeutic agent to a desired site, e.g. tumor, and providing a therapeutic effect. Examples of targeting agents include agents which can carry toxins or other agents which provide beneficial effects. The targeting agent can be an antibody linked to a toxin, e.g. ricin A or an antibody linked to a drug.

Neurotransmitters are substances which are released from a neuron on excitation and travel to either inhibit or excite a target cell. Examples of neurotransmitters include dopamine, serotonin, q-aminobutyric acid, norepinephrine, histamine, acetylcholine, and epinephrine.

Cell response modifiers are chemotactic factors such as platelet-derived growth factor (PDGF). Other chemotactic factors include neutrophil-activating protein, monocyte chemoattractant protein, macrophage-inflammatory protein, platelet factor, platelet basic protein, and melanoma growth stimulating activity; epidermal growth factor, transforming growth factor (alpha), fibroblast growth factor, platelet-derived endothelial cell growth factor, insulin-like growth factor, nerve growth factor, and bone growth/cartilage-inducing factor (alpha and beta), or other bone morphogenetic protein.

Other cell response modifiers are the interleukins, interleukin inhibitors or interleukin receptors, including interleukin 1 through interleukin 10; interferons, including alpha, beta and gamma; hematopoietic factors, including erythropoietin, granulocyte colony stimulating factor, macrophage colony stimulating factor and

granulocyte-macrophage colony stimulating factor; tumor necrosis factors, including alpha and beta; transforming growth factors (beta), including beta-1, beta-2, beta-3, inhibin, and activin; and bone morphogenetic proteins.

VIII. Utilities/Formulations

Polymer networks of the present invention have a wide variety of uses. A number of applications for the responsive gels of the invention are listed in Gel Science, Inc. brochures "Gel Sciences, the leader in Engineered Response Gels", G001-2/94-10M; "Separations", S001-2/94-10M, and "Controlled Release", CR001-2/94-10M, which are incorporated herein by reference. These applications include: 1) Separations, or reduction in the solvent level, of water or reduction in the water level of a number of products including protein, food protein, other food components; 2) Medical, pharmaceutical and diagnostic applications including electrophoresis, iontophoresis, free drug assay, spinal fluid diagnostics, assay, blood ultracentrifugation, cell culturing, wound dressing, exudate absorption and bacterial indicators; and 3) Toys, in which the toy needs to be biologically inert and safe. Exemplary applications and/or formulations of the polymer gel networks of the present invention are discussed below:

A. *Separation of a Solvent from a Solute*

In food separations or pharmaceuticals, responsive gels of the present invention may be used to selectively incorporate a solvent from a solute or separate a protein (or a drug or other solute) from a solution. The polymer networks of the invention are thus generally applicable to any process of selectively excluding a solute from a solvent by selectively incorporating the solvent. The term "selectively incorporating" refers to procedures whereby all, or a portion, of a low molecular weight solvent (e.g. water) is selectively removed by a polymer network from a solution of a higher molecular weight solute (e.g. synthetic or natural polymers, organic compounds, proteins, suspended particles and the like). The term "high molecular weight solute" refers to solutes having a molecular weight of at least about 250. Solvent/solute systems that may be utilized in the present invention include systems in which solute

is dissolved and those in which solute is dispersed or suspended in solution. The polymer gel network does not necessarily incorporate the solute if the solute is large enough. The fluid remaining after sorption is concentrated with solute and may be removed.

A preferred separations process functions by first contacting a solvent and solute with a polymer gel network of the invention capable of selective incorporation of the solvent. The gel physically expands as the entire gel, or a sorptive component thereof, accumulates solvent within the interior of the gel. At least part of the solvent is thereby incorporated by the gel, but solute is excluded from entering the gel. After expansion of the gel, the concentrated solute external to the swollen gel is separated from the swollen gel by centrifugation, filtration, or other conventional methods. The expanded gel may be discarded. The gel may also be collapsed such that solvent is released. This "regeneration" step is preferred so that the solvent-incorporating polymer gel is returned to a condition where it is again available to selectively accumulate solvent (see, for example, Cussler U.S. Patent 4,555,344, incorporated herein by reference).

Polymer gels used in this method may be expanded by either (i) contacting a gel with a solvent containing a solute and allowing the gel to non-reversibly swell and selectively incorporate the solvent; (ii) initiating a reversible volumetric expansion of the gel to selectively incorporate solvent by triggering the expansion with a stimulus; or (iii) a combination of (i) and (ii). Expansion is particularly advantageous and energy efficient for initiating selective incorporation when a convenient environmental trigger is available. Solvent incorporated by polymer gels can preferably be disgorged by initiating a volumetric gel collapse. In preferred embodiments of the invention, therefore, a solvent-containing polymer gel of the invention is challenged with an environmental change (e.g. pH; see Example 1), and the environmental change affects the gel by causing the entire gel, or a component thereof, to undergo a collapse. The collapsed polymer gel can then be separated from the disgorged solvent by, for example, filtration and/or centrifugation. Reversible collapse of the polymer gels is particularly useful for regenerating the gel because, after the polymer gel is collapsed, it may be re-expanded. The solvent released

during this regeneration may be recycled from the system or discharged as waste solvent.

B. *Separation of a Solute from a Solvent*

In an analogous manner, responsive gels of the present invention may be used to selectively incorporate a solute from a solvent to separate a protein (or a drug or other solute) from a solution. The polymer networks of the invention are thus generally applicable to any process of selectively excluding a solute from a solvent by selectively incorporating the solute. A preferred separations process functions by first contacting a solvent and solute with a polymer gel network of the invention capable of selective incorporation of the solute. Typically, the gel will contain an immobilized ligand that will form a binding pair with the solute of choice. The gel physically expands as the entire gel, or a sorptive component thereof, accumulates and binds solute within the interior of the gel. At least part of the solute is thereby incorporated by the gel. After expansion of the gel, the concentrated solvent external to the swollen gel is separated from the swollen gel by centrifugation, filtration, or other conventional methods.

i. Lipid Scavenging

In one particular method of interest, ligands that scavenge lipids are attached to KATP gels of the invention and are used to reduce the level of undesirable lipids present in the gastrointestinal tract of an animal. KATP cellulose ethers containing multiple reactive hydroxyl groups are particularly suitable for this purpose since the ligands may be bound thereto via stable ether linkages. Ligands for scavenging lipids are generally hydrophobic, capable of being attached to the polymer gel and may range from 1 to about 50% of ligand groups relative to the polymer. They are exemplified by straight chain aliphatics between C_{12} and C_{24}, as well as cholesterol itself. Hydroxyl groups of ligands may be converted to epoxy groups for reaction with KATP cellulose ether polymers of the invention. Depending on the particular ligand chosen, lipids which can be removed include cholesterol, cholesterol esters, steroids, fat soluble drugs, fatty acids and fatty acid esters (see, for example,

Nightingale et al. Future Perspectives of Biomedical Polymers, Dec. 4-6, 1992, Maui, HI).

C. *Protection of a Compound's Activity*

In another method of interest, immobilization and protection of a catalyst such as an enzyme within a responsive KATP gel enables the immobilized enzyme to be active and effective in environmental conditions in which the gel is expanded (see Example 1). Changing the environment (e.g. lowering pH) shrinks the gel and inactivates the enzyme by shutting off accessibility of the reactants in the solution to the catalyst.

D. *Delivery of a Compound into an Environment*

The polymer gel networks of the present invention can be used to deliver a compound (e.g. a drug) into an environment.

Drug delivery from acrylate-based hydrogels has been described by Kou et al. (J. Control Release 12:241, 1990). In one embodiment of the present drug delivery method, a KATP responsive gel is loaded with a biologically active compound at one temperature and induced to undergo a volumetric collapse to disgorge the entrained biologically active compound at another temperature. Delivery of the compound may be modulated by a temperature higher than the temperature of the gel in its loading mode (see, for example, Gutowska et al. J. Control Release 22:95, 1992: using NIPA to release heparin at high temperature).

In another embodiment, a microporous KATP responsive gel is loaded with a biologically active compound at one temperature and induced to undergo a volumetric expansion at another temperature to allow fluid from the environment of use (e.g. blood, lymph) to enter the expanded gel and biologically active compound to exit the expanded gel via diffusion through the pores. In a preferred drug delivery embodiment, the gel expands to release a drug during exposure to pH conditions that are different than the pH conditions to which it is exposed in the loading mode.

Polymer gel networks of the present invention can be prepared in any of a variety of different drug-delivery formulations, depending on the mode by which the

compound is to be delivered. The different formulations include, but are not limited to, those suitable for oral delivery, mucosal delivery, nasal delivery, ocular delivery, vaginal delivery, rectal delivery, dermal delivery (e.g. transdermal delivery), and internal delivery. Formulations can be prepared that are suitable for delivery to humans, animals (including mammals, birds, reptiles, fish, insects, and arachnids), or plants. Particular examples of such different formulations are discussed individually below. The descriptions presented below are not intended to be limiting of the present invention, but rather are intended to exemplify the advantages of the safe polymer gel networks of the present invention for use in delivering compounds to an environment.

i. Oral Delivery Formulations

Polymer gel compositions of the present invention are particularly useful for oral delivery compositions. For example, polymer gel networks of the present invention that are responsive to changes in pH can be utilized to effect controlled release of compounds at specific locations along the gastro-intestinal tract. Similarly, polymer gel networks that are responsive to changes in pH can be utilized, for example, to effect controlled release of substances into only one of a cow's stomachs.

Without wishing to be bound by any theory, a cellulose ether gel such as HPC with an LCST near body temperature (e.g. 42° C) should have its LCST shifted to a lower temperature at lower pH. This is because very few -COOH and/or -OH groups are ionized at low pH and the gel would tend to have a reduced hydrophilicity. At higher pH, many -COOH and/or -OH groups will be ionized and the LCST is shifted to a higher temperature due to increased hydrophilicity. Around body temperature, the gel is therefore very sensitive to pH change and would be collapsed at low pH (i.e. that of the stomach, where the drug would be retained within the polymer network) and expanded at higher pH (i.e. that of the intestine, where the polymer gel network would expand and release the drug). A responsive gel may be made from starting materials (i.e. cellulose ethers of various configurations) that vary in their hydrophobic/hydrophilic nature when ionized, so that the methods described herein may be used to make a reversibly responsive, pH-sensitive gel with an LCST designed

to match the body temperature of a desired subject. The LCST of preferred cellulose ethers is well known and can be easily determined and verified. Exemplary LCST's (°C) are 49° (MEC); 42°-46° (HPC); 59° (methyl(hydroxypropyl)cellulose: HPMC); 60° methyl(hydroxyethyl)cellulose; and 55°-70° (ethyl(hydroxyethyl)cellulose).

Polymer gel compositions that are responsive to changes in other environmental parameters can also be utilized in controlled release oral delivery formulations of the present invention. For example, polymer gel networks that are responsive to a magnetic or electric field can be used to specifically release a compound at a desired location or time. One particular application for such a composition of the present invention is in the delivery of fertility drugs (e.g. hormones) to fish. In fisheries, it is desirable for hormones to be administered to all female fish at the same time, so that fertilization can be performed coordinately in a single location. The present invention offers oral delivery compositions composed of responsive gel networks that, for example, collapse in response to the introduction of a magnetic field (see, for example, USSN 08/393,971, filed February 24, 1995, incorporated herein by reference). Fish fertility hormones can be incorporated into such compositions, which can then be administered to fish. Once all fish have had a chance to ingest the compositions, release of the incorporated hormone can be coordinately induces by application of a magnetic field.

Many different formulations that are useful for delivery of biologically active compounds to humans or animals are known in the art such as, for example, tablets, capsules, lozenges, pumps, etc., including osmotically controlled systems (see, for example, Theeuwes *Drug Absorption* Prescott et al. (eds) ADIS Press, 157, 1987; Eckenhoff et al. U.S. Patent No. 4,539,004; Eckenhoff et al. U.S. Patent No. 4,474,575). The present invention provides improved versions of each of these oral delivery formulations, because utilization of responsive gel polymers allows controlled substance release, and also offers protection of substance activity until the point of release.

In one particular oral delivery formulation of the present invention, a coated tablet is provided in which a biologically active compound is incorporated within a tablet that is substantially coated with a responsive polymer gel network of the present

invention. Such a coated tablet of the present invention provides for protection of the biologically active compound until the point of release, and for release of the biologically active compound in response to a predetermined change in environmental condition (e.g. pH, magnetic field, electric field, electromagnetic radiation, etc.).

Present-day controlled release coated tablets rely on differing solubilities of coating components to regulate release of encapsulated materials. Specifically, controlled-release tablets are avaiiable that are coated with a membrane that is punctuated with soluble components (see, for example, Healey "Enteric Coatings and Delayed Release" in *Drug Delivery to the Gastro-Intestinal Tract* Hardy et al. (eds) Ellis Horwood, Inc., Ch. 7, 1989). At a certain point, the soluble components dissolve and the tabletted material is release through the resultant pores in the membrane. One disadvantage with this system is that, although release of the tabletted material is delayed, it is not really "controlled" in the sense that it cannot be predicted at exactly what point the soluble plugs will have dissolved. Coated tablets of the present invention offer improved controlled release properties.

Another preferred oral delivery formulation of the present invention utilizes responsive polymer gel networks in pump-type delivery systems that are somewhat analogous to present-day osmotically-controlled oral delivery systems. Present-day osmotically-controlled oral delivery systems include the OROS® and OSMET® systems from Alza Corporation (Palo Alto, CA; see, for example, Eckenhoff et al. U.S. Patent No. 4,474,575; Eckenhoff et al. U.S. Patent No. 4,539,004, each of which is incorporated herein by reference).

Figure 3 presents various embodiments of present-day osmotically-controlled oral delivery systems. In one embodiment, presented in Figure 5A, one side of the osmotic delivery device housing **100** is comprised of a semi-permeable membrane **110**. The side opposite the semi-permeable membrane **110** has a delivery orifice **120**. A moveable partition **130** positioned inside the housing separates a drug compartment **140** from an osmotic agent compartment **150**. When the device is delivered to a subject, the osmotic agent expands and pushes the moveable partition **130** toward the delivery orifice **110** so that drug located in the drug compartment **140** is pushed out through the delivery orifice **110** and into the subject.

The present invention provides a somewhat analogous system, in which the osomotic agent is replaced by a crosslinked polymer gel network having a suitable KATP. Preferably, the polymer gel network is a responsive polymer gel network, and drug is delivered not by osmotic action, but as a result of the responsive polymer gel network expanding in response to a change in environmental condition. Such an oral delivery device provides advantages over the osmotically-controlled oral delivery devices because it provides increased control over the timing of drug delivery. For example, in particularly preferred embodiments, the polymer gel network expands in response to a change in pH, and the drug is not delivered until the device passes through the portion of the gastrointestinal tract in which the appropriate pH is achieved. In most preferred embodiments, the polymer gel network comprises a crosslinked polysaccharide gel network (e.g. a cellulosic gel network such as those described in Examples 1-11). This type of oral delivery system of the present invention is particularly useful for delivery of insoluble agents such as, for example, carbamazepine, phenytoin, griseofulvin, cyclosporine, etc.).

Another embodiment of a present-day osmotically-controlled oral delivery system is presented in Figure 3B. This embodiment is useful for delivery of liquid samples. In this embodiment, the housing **100** is comprised of a rigid, semi-permeable membrane. The osmotic agent forms a layer **160** inside the housing and surrounding the drug compartment **140**. A flexible, impermeable reservoir wall **170** lines the drug compartment **140**, and separates it from the osmotic agent. A delivery tube **180** is positioned inside the drug compartment so that it provides a passageway to the external environment, by means of the delivery orifice **110**. When the device is delivered to a subject, the osmotic agent swells so that the volume of the drug compartment **140** is reduced, and the liquid sample is pushed through the delivery tube **170**, out the delivery orifice **110**. Once again, the present invention provides a device that is somewhat analogous to the device depicted in Figure 3B, differing in that the osomotic agent depicted in Figure 3B is replaced by a crosslinked polymer gel network having a suitable KATP.

Yet another embodiment of a present-day osmotically-controlled oral delivery system is presented in Figure 3C. In this embodiment, the housing **100** is comprised

of a semi-permeable membrane that defines a single compartment **145** with a delivery orifice **110**. Both the osmotic agent and the rdug are disposed within the compartment **145**. When the device depicted in Figure 3C is delivered to a subject, gastro-intestinal juices permeate the compartment **145**, so that the osmotic agent is caused to swell. The increase in osmotic pressure results in expulsion of the drug through the delivery orifice **110**. The improvement of the provided by the present invention is the use of a safe polymer gel network instead of an osmotic agent. Preferably, a safe, environmentally responsive, gel network is utilized. Most preferably, the polymer gel network comprises a crosslinked polysaccharide gel network (e.g. a cellulosic gel network such as those described in Examples 1-11).

ii. Mucosal Delivery Formulations

Preferred mucosal delivery systems of the present invention are bioadhesive systems utilizing safe polymer gel networks of the present invention. The term "bioadhesive", as used herein, refers to the property, displayed by certain compounds, of showing an affinity for biological tissue. Example 19 describes preferred bioadhesive polymer gel networks of the present invention, that can effectively be utilized in mucosal delivery fomrulations to effect controlled drug delivery to mucosal tissues.

iii. Ocular Delivery Formulations

Many different kinds of ocular preparations incorporating gels, or gelling materials, are known in the art (see, for example, Davis et al. U.S. Patent No. 5,192,535; Missel et al. U.S. Patent No. 5,212,162; Joshi et al. U.S. Patent No. 5,252,318; Viegas et al. U.S. Patent No. 5,300,295; Haslam et al. U.S. Patent No. 4,474,751; Jani et al. U.S. Patent No. 4,911,920; Katz U.S. Patent No. 4,343,787). The present invention provides improved gel compositions for ocular delivery applications. For example, the present invention provides sustained delivery gel compositions in which release of a drug incorporated into the gel matrix occurs over a relatively long period of time (see, for example, Example 12).

The present invention also provides ocular delivery formulations comprising responsive gel compositions, so that delivery of a desired substance can be initiated in response to a change in environmental condition.

Safe polymer gel networks loaded with a desired biologically active compound (e.g. an ophthalmic diagnostic agent or surgical additive, an anti-glaucomal, anti-viral, or anti-microbial compound, a lubricant, etc.) can be incorporated into contact lenses, or other ophthalmic compositions according to methods known in the art, and can subsequntly be brought into contact with the eye of a subject, so that the desired biologically active compound is released in a sustained manner and/or in response to a particular change in environmental condition.

In a preferred ocular delivery formulation of the present invention, a lightly-crosslinked safe polymer gel of the present invention is utilized as a lacrimator. Such a composition has advantages over eye drops that are currently used as lacrimators because, by virtue of being lightly crosslinked, it is more viscous than an eyedrop and is not immediately cleared from the eye. Particularly preferred compositions are bioadhesive (see, for example, Example 19).

iv. Vaginal Delivery Formulations

Safe polymer gel compositions of the present invention can be utilized for vaginal delivery of biologically active compounds.

In one particular vaginal delivery formulation of the present invention, a pH-responsive polymer gel network loaded with an anti-fungal compounded is prepared and utilized for treatment of yeast infections. Infection of the vaginal tract with yeast results in alkalinization of the environment. A responsive polymer gel network can be prepared according to the principles and procedures described herein to release an antifungal compound in response to the increase in pH. As the infection is brought under control, the pH is decreased, and the responsive polymer gel network ceases to deliver more drug. The present invention provides controlled release compositions that are engineered so that drug is delivered only when it is needed, i.e. when the pH is elevated.

Another embodiment of a vaginal delivery formulation of the present invention utilizes a safe polymer gel network to deliver water to a dehydrated vagina. Post menopausal women, and also women who are breast feeding, often suffer from vaginal dryness that stems from atrophy of vaginal mucosa as a result of increased estrogen levels (see, for example, Gass et al. Compr. Ther. 16:3, 1990; Sarazin et al. Nurse Pract. 16:30, 1991). Safe polymer gel networks of the present invention can be utilized to deliver water to a dry vaginal, either by, for example, simple desorption, or, in the case of compositions utilizing responsive gel networks, in response to a predetermined change in an environmental condition.

Yet another embodiment of a vaginal delivery formulation of the present invention comprises a safe polymer gel network loaded with a spermicide (and/or a viricide). Such a contraceptive vaginal delivery formulation can offer advantages over present-day contraceptive devices, because they can be engineered, according to the principles and procedures set forth herein, to release the spermicide and/or viricide in response to a particular, predetermined, change in environmental condition.

Still another embodiment of a vaginal delivery formulation of the present invention comprises a safe polymer gel network loaded with a vaccine, for example to protect against a sexually transmitted disease (e.g. a herpes virus or an immunodeficiency virus; see, for example, Mark et al. Science 260:1323, 1993, incorporated herein by reference).

v. Rectal Delivery Formulations

Safe polymer gel networks of the present invention can readily be prepared in suppository formulations to allow delivery of biologically active compounds through the rectum. Rectal delivery allows increased bioavailability of delivered compounds (at least in part because the compounds do not encounter degredative enzymes etc. found in the gastrointestinal tract), and is particularly useful for deliver of high molecular weight compounds.

vi. Dermal Delivery Formulations:

Polymer gels of the present invention incorporating, for example, a medicament like hyaluronic acid, may be incorporated into a bandage, gauze or other conventional wound dressing, to allow dermal delivery of the medicament. For example, responsive gels can be incorporated into a dermal delivery formulation, so that, upon activation by an appropriate environmental trigger such as a temperature change or a change in the energy of incident light, the responsive gel collapses and disgorges the entrained medicament to the wound environment. If the gel is triggered to expand and release the medicament, it may also incorporate wound exudates during the expansion (see, for example, U. S. Patent 4,659,700, incorporated herein by reference).

Polymer gel compositions can also be incorporated into transdermal devices, and, in preferred embodiments, can be formulated into a novel, bi-layer transdermal device. Present-day transdermal devices are tri-layer devices, comprising a backing, a reservior (comprising the drug to be delivered in some sort of matrix), and a membrane. The membrane provides the rate-limiting step in drug delivery, and also typically has bioadhesive characteristics so that the transdermal device has an affinity for skin. THe transdermal device is positioned on skin so that the membrane is in contact with the skin, and the drug is delivered to the skin by passing through the membrane. The flux of the drug through the skin (J) is approximated by the following equation:

(Equation 6) $J = D(C_{patch}-C_{blood})\Delta X$,

where D is the diffusion coefficient of the drug through the skin, C_{patch} is the concentration of drug in the transdermal device, C_{blood} is the concentration of drug in the blood, and ΔX is the thickness of the skin. Because C_{blood} is effectively zero before the transdermal device is applied, the equation can be simplified to:

(Equation 7) $J = DC_{patch}/\Delta X$,

so that the flux, J, is determined by the concentration of drug in the device and a higher concentration gives higher flux.

The present invention provides an improved, bi-layer, transdermal device in which the drug is loaded into a polymer gel network, which is applied to a backing. Preferably, the polymer gel network is a responsive polymer gel network, and no rate-limiting membrane is required. In particularly preferred embodiments, the polymer gel network is also bioadhesive (see Example 19).

Polymer gel networks of the present invention can be loaded with very high levels (exceeding 20% by weight) of a biologically active compound (i.e. a drug; see above and see also USSN 08/276,462, filed July 18, 1994 and incorporated herein by reference). In preferred embodiments of the transdermal device of the present invention, the polymer gel network is loaded with at least about 20% by weight of a biologically active compound. Such a preferred transdermal device of the present invention offers increased flux (relative to current transdermal devices) of biologically active compound across the skin, due to the increased loading of the drug into the polymer gel network.

Iontophoretic devices made of KATP polymers are also within the scope of the invention. Iontophoretic function of a KATP polymer gel network of the invention may conveniently be studied *in vitro* in a commercially available Franz-type transport cell. A KATP polymer gel of the invention is loaded with a drug according to any procedure, preferably those described herein. The loaded gel is placed in the reservoir of a well type electrode. The upper (donor) portion of the cell is separated from the buffer-filled bottom (receptor) portion by a membrane (e.g. porcine skin or a synthetic membrane). In a typical protocol, current is applied to the anode which drives the positively charged drug through the membrane into the receptor solution. The amount of drug in the receptor solution is assayed using, for example, HPLC (see, for example, U.S. Patent 4,141,359, incorporated herein by reference).

In preferred iontophoretic devices of the present invention, a biologically active compound is loaded into a safe polymer gel network that is responsive to the application of an electric field. In particularly preferred embodiments, delivery of the drug is coordinated with the volume change (e.g. collapse) of the gel, and the drug is not released from the device until exactly the time that the electric field is applied to encourage the drug through the dermis.

vii. Internal Delivery Formulations:

Safe polymer gel compositions of the present invention can also be utilized in internal delivery formulations, for example to allow controlled release of biologically active compounds that are desirably administered at regulated times. "Internal delivery formulations" are those that allow for delivery of a compound within the body of a subject, and can include oral delivery formulations, injectable formulations, implantable formulations (e.g. transdermals), etc. Internal delivery formulations discussed in this section are those that are not specifically addressed elsewhere in the present specification. In one example of an internal delivery formulation of the present invention, compositions are provided that can be implanted in the body and activated by an externally-controlled source (e.g. a magnetic field, an electric filed, pressure, etc.) to release, for example, a pain-killer, only when it is specifically required. Such compositions can also be used for controlled release of compounds that are administered cyclically, at predetermined intervals (e.g. insulin, hormones, nitroglycerin, compounds whose administration is related to circadian rhythms, etc.).

Safe polymer gel compositions of the present invention can be utilized to deliver any biologically active compound internally. In particularly preferred embodiments, internal vaccine delivery compositions are provided, formulated, for example, for delivery by injection or by ingestion.

viii. Agricultural Delivery Formulations

Safe polymer gel compositions of the present invention are also desirably prepared in formulations designed for agricultural delivery. One of the problems associated with delivery of agricultural products (e.g. fertilizers, herbicides, etc.) is that many of the compounds utilized pose health hazards to the workers responsible for applying them. Incorporation of the hazardous compounds into safe polymer gel networks of the present invention avoids exposure of handlers to the chemicals. In preferred embodiments, the compounds are incorporated into responsive gel networks, and are released in response to a stimulus (e.g. magnetic field, magnetic field, rain, sunshine, pH, etc.).

ix. Medical Instruments

Electrodes and other monitoring instruments may have polymer gels of the present invention incorporated within or on the electrode. The gel may have materials such as ligands, enzymes, and the like, immobilized in or on the gel network (see, for example, U.S. Patent 4,274,420, incorporated herein by reference).

Polymer gels of the present invention can also be incorporated into or onto other medical instruments such as, for example, the tip of a balloon catheter, a stent, an intervertebral disc nucleus, etc. The polymer gel network need not coat the entire surface of the device. Loading of a drug into, a safe polymer gel network that is incorporated into or onto a medical device can protect the activity of the drug until it is delivered, at the desired time and location. For example, it might be desirable to deliver a compound that dissolves blood clots into a clogged artery at the point of obstruction. Such a compound can be incorporated into a safe polymer gel network, and preferably a responsive polymer gel network and affixed, for example, to the tip of the balloon catheter. When the catheter is in position adjacent the obstruction, release of the compound can be triggered by application of the appropriate environmental stimulus (e.g. heat, light, magnetic field, mechanical force, etc.). Alternatively or additionally, it might be desirable to introduce an anti-cogulant (e.g. heparin) to reduce the likelihood of a thrombolytic event during a surgical procedure.

Additional advantages associated with coating medical devices with polymer gel compositions of the present invention is that the polymer gel compositions can provide cushioning and or increased lubricity to the coated instruments.

In one embodiment of a coated medical device of the present invention, a safe polymer gel network of the present invention is formed, through the production of "polyelectrolyte complexes" on the surface of a medical device (see, for example, Examples 15-17). A drug can be loaded into the gel by any available method including those described herein. Preferably, the drug is loaded into the gel when the gel is swelled in the presence of the drug. The coated medical device is then positioned in or on a subject, and the drug is delivered to the subject. In some cases, the safe polymer gel network that is coated on the device is a responsive polymer gel network, and the drug is delivered to the subject when the gel in response to an

environmental stimulus. In other cases where the safe polymer gel network is a responsive polymer gel network, the drug is delivered when the gel network is expanded, so that pores open in the gel network and the drug escapes. One of ordinary skill can readily select an appropriate type of gel, with desired responsive characteristics, depending on the type of drug to be delivered, the type of environment into which the drug is to be delivered, the length of time over which the drug is to be delivered, etc.

It is not necessary that the safe polymer gel networks used to coat medical devices be responsive polymer gel networks. For example, a drug can be loaded into a non-responsive polymer gel network on the surface of a medical device, and can be encapsulated therein, so that the drug is maintained within the polymer gel until the encapsulation is broken by, for example, mechanical force or other means (see, for example, Example 18).

ix. Cell Culturing Formulations

Safe, responsive, polymer gel networks of the present invention can also be prepared in cell culturing formulations, for use as a bed material from which cultured cells are readily released. In current cell-culturing systems, cultured cells are typically collected or detached from the material on which they are cultured through proteolytic (e.g. with trypsin) or chemical treatment (e.g. with EDTA). Recently, an environmentally-responsive cell culturing material has been described, from which cells can be released by inducing collapse of a poly(N-isopropylacrylamide) gel (see, for example Okano et al. U.S. Patent No. 5,248,766; Okano et al. J. Biomed. Mat. Res. 27:1243, 1993; Yamada et al. Makromol. Chem., Rapid Commun. 11:571, 1990, each of which is incorporated herein by reference). Although this recently-reported cell-culturing system is interesting, its usefulness is limited because the gel material utilized is poly(N-isopropylacrylamide), a compound with known toxicities to animal cells. There is a need for an improved cell culturing matrix that utilizes safe polymer gel networks.

The present invention provides exactly such an improved, safe, cell culturing material. Essentially, a safe polymer gel network of the present invention, and

preferably a safe, responsive polymer gel network of the present invention, is physically and/or chemically coated on a solid support (e.g. a petri dish, a plate, a fiber, etc.). Cells are cultured on the polymer gel material, in the presence of any necessary and/or desirable nutrients.

The cell culturing material of the present invention can be utilized in either of two distinct ways. First of all, cells can be cultured on a shrunked, or collapsed gel, and then can be released from the gel when the gel expands (see Okano et al., Okano et al., and Yamada e al., supra). For example, in preferred embodiments, the safe polymer gel utlized in the cell culturing system iof the present invention is a respnsive gel. In the case where the gel is responsive to temperature (as is the case for HPC and HPMC gels, for example), cells are cultured on the polymer gel netowrk at a temperature above the LCST of the network, so that the network is collapsed. After a period of time, the temperature is decreased to a point below the LCST, so that the gel expands and the cells are released.

In alternate embodiments of the cell culturing system of the present invention, cells are cultured on an expanded responsive gel, and cell release is induced by triggering collapse of the gel network in response to the appropriate environmental stimulus (see Okano et al., Okano et al., and Yamada e al., supra).

E. *Pharmaceutical Compositions*

Polymer networks and biologically active compounds that are incorporated in, or on, the network may be used in pharmaceutically-effective amounts, with or without a compatible carrier. The term "carrier" includes any liquid, gel, fluid, ointment, cream, lotion or the like, which is suitable for use in, or on a subject and which does not interact with the other components of the polymer network in a deleterious manner. The term "compatible", as used herein, means that the components of the pharmaceutical compositions are capable of being commingled with the polymer network of the present invention, and with each other, in a manner such that there is no interaction which would substantially reduce the pharmaceutical efficacy of the pharmaceutical. A "pharmaceutically-effective amount" of a biologically active material or polymer network containing the material is that amount

which produces a result or exerts an influence on the particular condition being treated.

Some examples of substances which can serve as pharmaceutically-acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives, such as methylcellulose, hydroxypropyl-methyl-cellulose, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; talc; stearic acid; magnesium stearate; calcium sulfate; vegetable oils such a peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; sugar; alginic acid; pyrogen-free water; isotonic saline; phosphate buffer solutions; cocoa butter (suppository base); emulsifiers, such as the TWEENs®; as well as other non-toxic compatible substances used in pharmaceutical formulations. Wetting agents and lubricants such as sodium lauryl sulfate, as well as coloring agents, flavoring agents, excipients, tabletting agents, stabilizers, antimicrobials, antioxidants, and preservatives, can also be present. Other compatible pharmaceutical additives and actives (e.g. NSAID's; pain killers; muscle relaxants) may be included in the pharmaceutically-acceptable carrier for use in the compositions of the present invention. For example, local anesthetics (e.g. benzyl alcohol; lidocaine) may be included in the pharmaceutically-acceptable carrier. Adhesive formulations may also be incorporated into the polymer gels of the invention. Exemplary adhesive devices are described in U.S. Patents 3,972,995 and 4,593,053, incorporated herein by reference.

The formulations include, but are not limited to, those suitable for oral, buccal, rectal, topical, nasal, ophthalmic (for example, see U.S. Patent 2,976,576 to a contact lens composition, incorporated herein by reference) or parenteral (including subcutaneous, intramuscular and intravenous) administration, all of which may be used as routes of administration for practicing the present invention. Other suitable routes of administration include intrathecal administration directly into spinal fluid (CSF), direct injection onto an arterial surface to prevent re-stenosis, and intraparenchymal injection directly into targeted areas of an organ.

Formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets, tablets or lozenges, each containing a predetermined amount of the potentiating agent as a powder or granules; as liposomes containing a loaded gel; or as a suspension in an aqueous liquor or non-aqueous liquid such as a syrup, an elixir, an emulsion or a draught.

Formulations suitable for parenteral administration conveniently may comprise a sterile emulsion or a sterile aqueous preparation of the active compound, which is preferably isotonic with the blood of the recipient.

Nasal spray formulations comprise purified aqueous solutions of the active compound with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes.

Formulations for rectal administration may be presented as a suppository with a suitable carrier such as cocoa butter, or hydrogenated fats or hydrogenated fatty carboxylic acids.

Ophthalmic formulations can be prepared by a similar method to the nasal spray, except that the pH and isotonic factors are preferably adjusted to match that of the eye.

In addition to the aforementioned ingredients, the formulations of this invention may further include one or more accessory ingredient(s) selected from diluents, buffers, biocides (e.g. chlorhexidine gluconate, triclosan, povidine-iodine, and the like), adhesives (e.g. lectin, pectin, fibronectin, and the like), flavoring agents, binders, anti-microbials, skin permeation enhancers, disintegrants, surface active agents, thickeners, lubricants, preservatives (including antioxidants), surfactants, and the like.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to known methods using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the

acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono-or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectibles.

F. *Cosmetic Compositions*

The KATP polymer gels of the present invention may be fabricated into a cosmetic compositions by combining the gel with fragrance or other cosmetic material and incorporate the gel into a cosmetic carrier. The cosmetic carrier may take the form of fatty or nonfatty creams, milky suspensions or emulsion-in-oil or oil-in-water types, lotions, gels or jellies, colloidal or noncolloidal aqueous or oily solutions, pastes, aerosols, soluble tablets or sticks. Generally, the carrier contains from about 0.001% to about 10% by weight of the gel of the invention. Preferred ranges are about 0.1% to about 10%.

Cosmetic compositions according to the invention may also combined with surface active agents of the anionic, cationic or nonionic type, emulsifying agents, perfumes, solvents, fats, oils and mineral wax, fatty acids and derivatives thereof alcohols and derivatives thereof, glycols and derivatives thereof, glycerol and derivatives thereof, lanolin, beeswax, oleic acid, spermaceti, almond oil, castor oil, sorbitol and derivatives thereof, tragancanth gum, clay, magnesia, talc, metal stearates, chalk, magnesium carbonate, and the like. These materials are well-known in the cosmetic art and are discussed, for example, in Remington's *Pharmaceutical Science*, McCutcheon's *Detergents*, and Sagarin's *Science and Technology of Cosmetics*, each of which is incorporated herein by reference. Exemplary cosmetic compositions used according to the present invention are given in Example 10. The cosmetic compositions used in the method according to the invention may also contain agents such as antibiotics, anti-inflammatories or anesthetics such as carbenicillin, chloramphenicol, gentamicin, penicillin G, polymyxin B, streptomycin, sulfacetamide, trifluridine, acyclovir, sulfadiazine, corticosteroids, nystatin, and miconazole.

The cosmetic compositions of the present invention may all contain various preservatives such as, butylated hydroxytoluene, methionine, cysteine, ascorbic acid, catalase, superoxide dismutase, glutathione, parabens and the like.

The invention will now be illustrated with the following, non-limiting examples.

IX. Examples

EXAMPLE 1: Preparation of pH and Temperature Sensitive Hydroxypropylcellulose Gel with Adipoyl Chloride Reagent

Exactly 50 mL of N-methyl pyrolidone (Fisher Scientific, Catalog No. 03688-4) was added to 5 grams of hydroxypropylcellulose (Aqualon, Klucel 99-EF NF). The two materials were mixed on a magnetic stirrer for about 2 hours, while covered, to achieve a clear and colorless solution. This solution was then placed in a refrigerator for about 1 hour in order to achieve a solution temperature of 4-8°C. To this solution, while stirring, 1 mL of cold (2-8°C) adipoyl chloride (Aldrich, Cat. No. 16,521-2) was added, and the resulting solution allowed to stir for 1 minute.

A microcapillary pipette of about 340 μm bore (Fisher Scientific, Cat. No. 21-16A-2A) was dropped into the solution. A gel formed in and around the pipette in about 3 hours. The pipette was then removed from the gel and placed in a vessel containing an excess of deionized water (Millipore Alpha-Q). After about 8 hours the water was decanted off, and the vessel filled with methanol (ACS grade). The pipette containing the gel was allowed to sit in methanol solution for 5 hours. This was followed by three more, 5 hour methanol washes.

The pipette was mounted in an airspace of a small, clear capsule (about 5cmx4cmx2cm). Temperature of the capsule was regulated by equilibrating it with well stirred, temperature controlled water solution.

A differential thermocouple arrangement permitted the monitoring of temperature differences between water and air within the capsule to about 0.005 deg. C. Water temperature within the capsule was measured to about 0.1 deg. C with a digital thermocouple (mfg. by Cole-Parmer Scanning Thermocouple Thermometer #92800-00).

Two sealed containers were partially filled with pure, degassed distilled water. One container also contained a port to allow addition of acid; the second container contained a port for addition of base. Use of a single container to generate a wide range of pH values from acid to base would lead to formation of neutral salt, which might have induced a volume change in the gel. A series of pH solutions were made, as described below, and then pumped through the bore of the tube at a flow rate of 3 mL/min.

The diameter of the gel cylinder was observed at each pH and recorded through the optically clear walls of the capsule using a 10X microscope. Volumetric ratio changes of the gel with pH were determined by cubing the ratio of the gel string diameter to pipette bore.

The pH solution was changed every 0.5 pH units and maintained to let the gel reach equilibrium. Then, the volume of the gel was measured. Water temperatures differed by no more than 0.1 °C during the experiments and was maintained at 25 °C.

Low pH values were obtained by adding concentrated hydrochloric acid in increasing amounts to the pure, distilled water in one container. Above the pH value for pure, distilled water lacking any acid addition (pH 6), the second container was employed and sodium hydroxide (1 N) was added. The pH was controlled by flowing dry nitrogen gas slowly through the headspace of each container to maintain a positive pressure and prevent entrance of ambient air into the container. The pH was recorded continuously in each container by an Orion combination pH electrode (#91-56) immersed in the solution connected to an Orion #520 pH meter.

This gel exhibited a volumetric dependency on pH illustrated in Figure 1.

EXAMPLE 2: Preparation of Hydroxypropylmethylcellulose Gel with Adipoyl Chloride Reagent

Exactly 50 mL of N-Methyl Pyrolidone (Fisher Scientific, Catalog No. 03688-4) was added to 5 grams of hydroxypropylmethyl cellulose (Dow Chemical, Methocel E5 PREM), and was mixed for 2 hours at 45°C, while covered, to achieve a clear straw-colored solution. This solution was then placed in a refrigerator for 1 hour in order to achieve a solution temperature of 4-8°C. To this solution, while stirring,

1 mL of cold (2-8°C) adipoyl chloride (Aldrich, Cat. No. 16,521-2) was added, and the resulting solution was allowed to stir for 1 minute. A gel formed in 12 hours. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of Methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel was both pH and temperature sensitive.

EXAMPLE 3: Preparation of Starch Gel with Adipoyl Chloride Reagent

Exactly 50 mL of N-Methyl Pyrolidone (Fisher Scientific, Catalog No. 03688-4) was added to 5 grams of Modified Food Starch (National Starch, #6818:77-3), and was mixed for 2 hours at 45°C, while covered, to achieve a clear straw-colored solution. This solution was then placed in a refrigerator for 1 hour in order to achieve a solution temperature of 4-8°C. To this solution, while stirring, 1 mL of cold (2-8°C) adipoyl chloride (Aldrich, Cat. No. 16,521-2) was added, and the resulting solution was allowed to stir for 1 minute. A gel formed in 12 hours. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water.

The pH responsiveness of this material was assayed by preparing the identical starch gel in pipettes according to the procedures of Example 1. The pH sensitivity was tested using the procedures and apparatus of Example 1 as well. Figure 2 illustrates the sharp volume transition over a small change in pH (less than 0.5 pH units).

EXAMPLE 4: Preparation of pH and Temperature Sensitive HPC Hydrogel

Exactly 50 mL of N-Methyl Pyrolidone (Fisher Scientific, Catalog No. 03688-4) was added to 5 grams of Hydroxypropylcellulose (Aqualon, Klucel 99-EF NF), and

was mixed for 2 hours at 45°C, while covered, to achieve a clear straw-colored solution. This solution was then placed in a refrigerator for 1 hour in order to achieve a solution temperature of 4-8°C. To this solution, while stirring, 1 mL of cold (2-8°C) sebacoyl chloride (Aldrich, Cat. No. 13,178-4) was added, and the resulting solution was allowed to stir for 1 minute. A gel formed in 12 hours. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. The pH responsiveness of this material was assayed by preparing the identical gel in pipettes according to the procedures of Example 1. The pH sensitivity was tested using the procedures and apparatus of Example 1 as well.

EXAMPLE 5: Preparation of pH and Temperature Sensitive HPC Hydrogel

Exactly 50 mL of N-Methyl Pyrolidone (Fisher Scientific, Catalog No. 03688-4) was added to 5 grams of hydroxypropylcellulose (Aqualon, Klucel 99-EF NF) and was mixed for 2 hours at 45°C, while covered, to achieve a clear straw-colored solution. This solution was then placed in a refrigerator for 1 hour in order to achieve a solution temperature of 4-8°C. To this solution, while stirring, 1 mL of cold (2-8°C) succinyl chloride (Aldrich, Cat. No. S645-2) was added, and the resulting solution was allowed to stir for 1 minute. A gel formed in 12 hours. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. The pH responsiveness of this material was assayed by preparing the identical gel in

pipettes according to the procedures of Example 1. The pH sensitivity was tested using the procedures and apparatus of Example 1 as well.

EXAMPLE 6: Preparation of Hydroxyethylcellulose Gel with Adipic Acid Reagent

Exactly, 45 mL of DMSO (Fisher Scientific, Catalog No. 03688-4) was added to a distillation flask containing 5 grams of hydroxyethylcellulose (Aqualon, Natrosol 99-250HBR PA) and 2 grams of adipic acid (Fisher Scientific, Catalog No. A44-500), and was mixed for 2 hours, while covered, to achieve a clear colorless solution. To this solution, 5 mL of toluene (Fisher Scientific), solution was added. This was followed by the addition of 500 μL of a 50% (v/v) solution of H_2SO_4 in DMSO. This solution was allowed to react under azeotropic distillation (see Haslam Tetrahedron 36:2409, 1980, incorporated herein by reference). After 12 hours, a gel formed in the flask. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel is not temperature sensitive but is pH sensitive.

EXAMPLE 7: Preparation of Hydroxyethylcellulose Gel with Citric Acid Reagent

Exactly 45 mL of DMSO (Fisher Scientific, Catalog No. 03688-4) was added to a distillation flask containing 5 grams of hydroxyethylcellulose (Aqualon, Natrosol 99-250HBR PA) and 2 grams of citric acid (Aldrich Chemical, Cat. No. 25,127-5), and was mixed for 2 hours, while covered, to achieve a clear colorless solution. To this solution, 5 mL of toluene solution was added, followed by the addition of 500 μL of a 50% (v/v) solution of H_2SO_4 in DMSO. This solution was allowed to react under azeotropic distillation (see Haslam Tetrahedron, 36:2409, 1980, above). After 12 hours, a gel formed in the flask. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours.

This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel is pH sensitive.

EXAMPLE 8: Preparation of Hydroxyethylcellulose Gel with 1,2,3,4-Butanetracarboxylic Acid Reagent

Exactly 45 mL of DMSO (Fisher Scientific, Catalog No. 03688-4) was added to a distillation flask containing 5 grams of hydroxyethylcellulose (Aqualon, Natrosol 99-250HBR PA) and 2 grams of 1,2,3,4-butanetracarboxylic acid (Aldrich Chemical, Cat. No. 25,730-3), and was mixed for 2 hours, while covered, to achieve a clear colorless solution. To this solution, 500 μL of a 50% (v/v) solution of H_2SO_4 in DMSO solution was added, followed by the addition of 5 mL of toluene. This solution was allowed to react under azeotropic distillation (see Haslam Tetrahedron 36:2409, 1980). After 12 hours, a gel formed in the flask. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel is pH sensitive.

EXAMPLE 9: Preparation of Hydroxyethylcellulose Gel with 1,10-Decandicarboxylic Acid Reagent

Exactly 45 mL of DMSO (Fisher Scientific, Catalog No. 03688-4) was added to a distillation flask containing 5 grams of hydroxyethylcellulose (Aqualon, Natrosol 99-250HBR PA) and 2 grams of 1,10-Decandicarboxylic Acid (Aldrich Chemical, Cat. No. D100-9), and was mixed for 2 hours, while covered, to achieve a clear colorless solution. To this solution, 500 μL of a 50% (v/v) solution of H_2SO_4 in DMSO solution was added, followed by the addition of 5 mL of toluene. This solution was allowed to react under azeotropic distillation (see Haslam Tetrahedron 36:2409, 1980). After 12 hours, a gel formed in the flask. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q).

After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel is pH sensitive.

EXAMPLE 10: Preparation of Hydroxyethylcellulose Gel with Sebacic Acid Reagent

Exactly 45 mL of DMSO (Fisher Scientific, Catalog No. 03688-4) was added to a distillation flask containing 5 grams of hydroxyethylcellulose (Aqualon, Natrosol 99-250HBR PA) and 2 grams of sebacic acid (Aldrich Chemical, Cat. No. 28,325-8), and was mixed for 2 hours, while covered, to achieve a clear colorless solution. To this solution, 500 μL of a 50% (v/v) solution of H_2SO_4 in DMSO solution was added, followed by the addition of 5 mL of toluene. This solution was allowed to react under azeotropic distillation (see Haslam Tetrahedron 36:2409, 1980). After 12 hours, a gel formed in the flask. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol (ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel is pH sensitive.

EXAMPLE 11: Preparation of Hydroxyethylcellulose Gel with Succinic Acid Reagent

45 mL of DMSO (Fisher Scientific, Catalog No. 03688-4) was added to a distillation flask containing 5 grams of hydroxyethylcellulose (Aqualon, Nitrosyl 99-250HBR PA) and 2 grams of succinic acid (Aldrich Chemical, Cat. No. 39,805-5), and was mixed for 2 hours, while covered, to achieve a clear colorless solution. To this solution, 500 μL of a 50% (v/v) solution of H_2SO_4 in DMSO solution was added, followed by the addition of 5 mL of toluene. This solution was allowed to react under azeotropic distillation (see Haslam Tetrahedron 36:2409, 1980). After 12 hours, a gel formed in the flask. The gel was then removed, cut into 1x1x1 cm^3 cubes, and placed in an excess of deionized water (Millipore Alpha-Q). After 12 hours the water was decanted off, and the vessel was filled with an excess of methanol

(ACS grade), and the cubes were allowed to sit in methanol solution for 12 hours. This was followed by 3 more 12 hour washes. The cubes were then dried in a desiccator, and then swollen in deionized water. This gel is pH sensitive.

EXAMPLE 12: Preparation of Cosmetic Composition

A gel containing a fragrance or an active ingredient according to the present invention includes the following materials:

a) distilled water:	65.1%;
crosslinked KATP hydroxypropylcellulose gel:	5.0%;
methylparaben:	0.17%;
propylparaben:	0.03%; and
b) polyoxethylene (20) sorbitan trioleate:	0.3%;
sorbitan monooleate:	0.15%;
caprylic/capric acid triglyceride:	2.5%; and
c) distilled water:	20.1%;
triethanolamine:	0.8%; and
d) active ingredient:	5.0%

Preparation of the gel is carried out as follows:

For obtaining a), the KATP responsive gel is expanded to incorporate the remaining components. Components of b) are also introduced by expanding the gel. The aqueous triethanolamine solution c) is added under stirring; finally, composition d) is added under stirring. Alternately, the gel is expanded to incorporate all the remaining components c) and d).

EXAMPLE 13: Loading and Release of Ovalbumin from HPC Gels

A series of experiments was performed to test loading and release of ovalbumin from HPC gels.

Synthesis of HPC Disks

A method of gel disc preparation has been described by Antonsen, et al. (K P Antonsen et al. Biomat, Art. Cells & Immob. Biotech 21:1, 1993). The crosslinking

reaction of HPC with adipoyl chloride was performed as described in Example 1. After the addition of adipoyl chloride, the mixture was poured into molds consisting of two glass plates separated by a 2-mm thick buna rubber spacer. The crosslinking was allowed to proceed for 24 hr at room temperature. After the crosslinking reaction, the gel sheet was cut into disks 12.5 mm diameter with a cork borer, and washed as in Example 1. The gels were then desiccant dried and weighed. These disks were pH and temperature sensitive.

Equilibrium Loading

The materials used were:

Buffer: KH_2PO_4/Na_2PO_4 (Buffer Salt, pH 6.86, Fisher Scientific, #B78).

Protein: Ovalbumin Grade II (A5253) Sigma Chemical (St. Louis, MO); 2.3 mg protein/mL soln.

Second Polymer: Polyvinyl Alcohol 87-89% hydrolyzed, Aldrich Chemical (36, 317-0) 10% by weight in loading soln.

Salt: No salt used

The loading of ovalbumin into the gel was performed by equilibrating the gel in an ovalbumin solution. Ten mL of PVA or ovalbumin/PVA solution were added to 20 mL glass vials into which the HPC gels were placed. One gel disc (3.5 mg total weight) were placed in each vial. The gels and vials were stored at room temperature in a desiccator jar and the weights of the gels were recorded vs time. A blot and dry method was used to weigh the gels. Once a constant weight was obtained, the gels were assumed to be equilibrated. Once equilibrated with the appropriate solutions, the gels were removed from solution and placed in a desiccator jar to dry.

The ovalbumin content was determined by mass balances. The amount of ovalbumin absorbed by the gel was assumed to be the difference between the dry loaded gel weight and the initial dry gel weight for gels loaded with, and without, PVA.

From mass balance calculations, the average percentage loading (n=3) of HPC gels with PVA, ovalbumin and buffer was 135.5% +/- 8.1%. The average percentage loading (n=3) of HPC gels without PVA was 38.3 % +/- 21.5%. The

estimated ovalbumin loaded is the difference between these numbers, or about 97 %. Thus, almost all of the ovalbumin was loaded into the HPC gels.

Release Kinetics

The ovalbumin released from the dry gels was determined as follows: The phosphate buffer solution was used to leach out the ovalbumin from the ovalbumin-loaded gels. Three mL of phosphate buffer was placed in glass vials. At specified time intervals, a defined volume of released solution was removed from the original solution. This volume of released sample was placed in a vial. Immediately after the removal of the released sample, an identical volume of fresh phosphate buffer was placed back into the original releasing media. Therefore, a constant volume was maintained for the release experiments. This process was continued for approximately 10 hours of regulated sampling, with samples taken every 20 minutes for the first 2 hours and then every hour. This particular technique allowed for assay of total amount of released ovalbumin.

Release of ovalbumin into phosphate buffer after 24 hr was equal to 8 mg, or 23 mg/mg dry weight gel.

EXAMPLE 14: Loading and Release of Amylase from HPC Gels

The loading of α-amylase into HPC hydrogels was performed by the same method as for ovalbumin with the following reagents:

Buffer: KH_2PO_4/Na_2PO_4 (Buffer Salt, pH 6.86, Fisher Scientific, #B78).

Protein: α-amylase, Bacillus subtilis; mol. wt. 48,450; Calbiochem 1,000,000 units (cat #171568); 1.37 mg amylase/mL, soln.

Second Polymer: PEG-PPG Copolymer (50/50 by weight), Pluronic P105, mol. wt. approx. 6,500 (BASF Performance Chemicals), 10% by weight in loading soln.

Salt: KI, ACS grade (Fisher Scientific, Cat. #P410), 0.22M.

Gels were loaded as above and then dried in desiccator at room temperature.

The release of α-amylase from HPC gels was performed by placing the dried gels in 3 mL release buffer (release buffer = 0.005 M KH_2PO_4, 0.005 M Na_2PO_4,

0.01 M $CaCl_2$) in glass vials. These vials were hand shaken initially and at various intervals during release. At timed intervals, the liquid was carefully removed from the vials and replaced with fresh buffer.

A bioactivity assay was performed using a Sigma Chemical Assay Kit #577 (based upon colorimetric measurement of the enzymatic release of p-nitrophenol from the substrate 4,6 ethylidene (G_1)-p-nitrophenol (G_1)-α, D-maltoheptaside). The concentration assay for amylase is run using a UV/VIS spectrophotometer (Shimadzu 160U) at 280 nm.
The bioactivity of the α-amylase was determined at selected intervals, and the concentration of the enzyme was assayed at all intervals.

Characteristic release curves for α-amylase from the HPC gel (not shown) revealed a diffusion-controlled release pattern with release as a function of the square root of time showing a linear relationship. The released enzyme maintained at least 40% of its original bioactivity over the release interval of 24 hours.

EXAMPLE 15: Forming a Safe Polymer Gel Network Comprising Polyelectrolyte Complexes on a Polyamide Device

In one embodiment of a coated medical device of the present invention a safe polymer gel network is formed on the surface of a device made from a polyamide material (e.g. nylon; see Figure 6). The polyamide material is treated with di- or multi-functional isocyanate, preferably according to procedures described by Fan (U.S. Patent No. 5,091,205, issued February 25, 1992, incorporated herein by reference), so that a reactive intermediate (i.e. an intermediate that contains isocyanato groups capable of further reaction-- e.g. polyurea) is formed on the surface of the material. The material is then treated with a polycarboxylic acid (e.g. polyacrylic acid, polymethacrylic acid, chondroitin-6-sulfate, etc.) under reaction conditions (see Fan, supra), so that the surface of the material now has a plurality of free carboxyl groups. The material is then exposed to one or more polycations (e.g. primary amines, secondary amines, tertiary amines, polyimines, basic polypeptides, and the like, including polyethyleneimine, polyethylenepiperazine, collagen), preferably in an aqueous solution. Interaction of the polycations with the free carboxylic acid groups

on the surface of the material results in the formation of "polyelectrolyte complexes" that comprise a safe polymer gel network of the present invention.

One of ordinary skill in the art can readily select particular di- or multi-functional isocyanates, polycarboxylic acids, and/or polycations so that the resultant polyelectrolyte complexes form a polymer gel network that undergoes a volume change in response to a change in environmental condition (e.g. mechanical force, pH, salt concentration/composition, etc.). Environmentally-triggered collapse or expansion of such polymer gel networks results in the network becoming less or more permeable to drugs.

EXAMPLE 16: Forming a Safe Polymer Gel Network Comprising Polyelectrolyte Complexes on a Polyethylene Device

In another embodiment of a coated medical device of the present invention a safe polymer gel network is formed on the surface of a device made from a polyethylene material (e.g. low density polyethylene; see Figure 5). The material is exposed to a polycarboxylic acid in the presence of irradiation energy, so that reaction between the polycarboxylic acid and the polyethylene results in free carboxylic acid groups extending from the surface of the polyethylene. For example, we reacted low molecular weight polyethylene with polyacrylic acid (10-30 w% aq) by exposure to γ-irradiation (e.g. by exposure to ^{60}Co; dose 0.5 Mrad; dose rate 100 rad/sec) in the presence of $FeSO_4(NH_4)_2SO_4$ (50 mM aq).

The material is then exposed to a polycation, so that a polyelectrolyte complex forms, as described in Example 15. We exposed the above-mentioned modified low molecular weight polyethylene material to polyethyleneimine (10-30 w% aq), so that polyelectrolyte complexes formed between the carboxylic acid groups on the surface of the modified low molecular weight polyethylene and the polyethyleneimine.

EXAMPLE 17: Coating a Device with a Safe Polymer Gel Network Comprising Polyelectrolyte Complexes

In yet another embodiment of a coated medical device of the present invention, a safe polyelectrolyte complex between a polyacid and a polybase is first formed in an

aqueous solution, and is subsequently coated onto a porous support (e.g. nitrocellulose acetate, cellulose acetate, polyethylene, polypropylene, teflon, etc.) by, for example, casting, precipitation, or impregnation (see Figures 6 and 7). If desired (e.g. for increased strength), a polycomplex between a polyacid comprising a polycarboxylic acid and a polybase comprising a polyamine can be crosslinked by intermolecular amide bonding.

We have coated a safe polymer gel comprising a collagen/chondroitin-6-sulfate (C6S) polyelectrolyte complex onto a polypropylene membrane by mixing collagen (3 g/L of 20 xx 1.5 μm particles) with C6S (0.2 g/L) for 2-4 hr at pH 7.2 and 4 °C (see Figure 7). The mixture was coated on a polypropylene membrane that had been pre-treated with methanol, and the coated membrane was then immersed in CH_3COOH (50 mM aq) for 1-2 hours, so that a polyelectrolyte collagen/C6S complex precipitated onto the membrane. The membrane was then placed on a steel surface which was cooled through contact with dry ice, so that the polyelectrolyte complex was frozen onto the membrane. Subsequently, the material was lyophilized (0.05 torr, 24 hours, room temperature, followed by 105 °C for 24 hours). Although it is not necessary, the material was subsequently immersed in glutaraldehyde (0.3 w%), in the present of aqueous CH_3COOH, at pH 3.5 and room temperature, for 24 hours. The resultant coated membrane was stable over a pH range of about 2 to about 10, and also at high ionic strength. Furthermore, the crosslinked polymer gel network was pH-responsive, and allowed regulation of the permeability of the polypropylene membrane (to drugs and/or other compounds) through controlled expansion or collapse of the gel.

EXAMPLE 18: Encapsulation of a Biologically Active Compound in a Safe Polymer Gel Network Coated on an Angioplasty Balloon

Angioplasty balloons are typically made of nylon, or another polyamide material. In one embodiment of a coated medical device of the present invention, such an angioplasty balloon is treated with an isocyanate, followed by a polycarboxylic acid, as described in Example 15 and in Fan (U.S. Patent No. 5,091,205). Angioplasty balloons coated with polyacrylic acid are currently available from Boston Scientific (Boston, MA). The coated balloon then immersed

in an aqueous solution of poly(ethylene glycol) (PEG) poly(ethyleneoxide) at pH ≤ 3.2. Due to hydrogen bond formation between electron deficient protons of the acid and electron-donating ether groups of PEG, a PEG/polyacid polymer gel network forms on the surface of the balloon. Of course, as would be apparent to one of ordinary skill in the art, other proton acceptor polymers (e.g. polypropylene glyocol, polypropylene oxide, poly (N,N-dimethylacrylamide)), or combinations thereof, could be used, provided that the final polymer gel network has an appropriate KATP.

In particularly preferred embodiments of the coated angioplasty balloon of the present invention, the polyacid-coated balloon is contacted with a drug prior to being immersed in the PEG solution, so that the drug is loaded into the polyacid gel, and in subsequently trapped therein by encapsulation with PEG. When the angioplasty balloon is inserted into a subject and inflated, the mechanical force exerted by the expanding balloon breaks the PEG encapsulation, so that pores open in the polyacid gel, and the drug is released.

EXAMPLE 19: Bioadhesive Safe Polymer Gel Compositions

Bioadhesive safe polymer gel compositions of the present invention have been formulated from a variety of different polymer starting materials, including Carbopol® 934P (polyacrylic acid: BF Goodrich), Carbopol® 974P (polyacrylic acid; BF Goodrich), Noveon® AA1 (polycarbophil; BF Goodrich), Natrosol® 250 HHX (hydroxypropylmethylcellulose; Aqualon), Natrosol® 250 HHR (hydroxypropylmethylcellulose; Aqualon), Methocel E10M (hydroxypropylmethylcellulose; Dow Pharmaceuticals), Methocel K15M (hydroxypropylmethylcellulose; Dow Pharmaceuticals), and Methocel K100M (hydroxypropylmethylcellulose; Dow Pharmaceuticals). In each case, the polymer was mixed using a mixer set at 800-1200 rpm. Deionized water was added until the polymer was solubilized. In some cases (e.g. Methocyl K4M), the deionized water was heated before being added. In some cases, 10% NaOH was added to raise the pH to allow gellation of polyacrylic acid polymer gels. Typically, the resultant gels contained 80-99.9% deionized water. The following Table presents the composition of some of the bioadhesive gel formulations:

WEIGHT % OF MATERIAL				
	Carbopol 974P Number 1	Carbopol 974P Number 2	Natrosol 250 HHR	Methocyl K4M
Polymer	0.5	10.0	2.0	2.0
DI water	97.5	90.0	98.0	98
10% NaOH	2.0	0.0	0.0	0.0

All of these bioadhesive gel formulations have a pH of about 5.0. Any of these bioadhesive formulations can be combined with other safe gel polymer networks (e.g. crosslinked polymer gel networks) of the present invention, for example so that the bioadhesive gel formulation comprises approximately 1-99% of the final gel composition (i.e. the gel composition comprising the bioadhesive gel formulation and another safe polymer gel netowrk of the present invention). Other materials, such as compounds that assist in dispersion of crosslinked polymer gel networks, can also be included in the final gel composition, which can then be loaded with a biologically active compound as described herein.

Equivalents

It will be understood that the preceding is merely a description of certain preferred embodiments of the present invention. It will be readily apparent to one of ordinary skill in the art that various modifications can be made without departing from

the spirit or scope of the invention. Modifications and equivalents are therefore within the scope of the invention.

Claims

What is claimed is:

1. A pharmaceutical composition for controlled delivery of a biologically active compound to a biological environment, comprising:

a polymer gel network having a known, acceptable toxilogical profile; and

an effective amount of the biologically active compound loaded in the polymer gel network.

2. The pharmaceutical composition of claim 1, wherein the polymer gel network has a composition selected so that the polymer gel network undergoes a volume change in response to a change in environmental condition.

3. The pharmaceutical composition of claim 2, wherein the change in environmental condition comprises a change in an environmental parameter selected from the group consisting of: temperature; pH; solvent composition; presence, energy, or orientation of an electric field; presence, energy, or orientation of a magnetic field; presence or magnitude of mechanical force; presence or energy of incident electromagnetic radiation.

4. The pharmaceutical composition of any of claims 1-3 wherein the polymer gel network has a composition selected so that the pharmaceutical composition is suitable for oral delivery.

5. The pharmaceutical composition of claim 4, wherein the polymer gel network has a composition selected so that the polymer gel network undergoes a volume change in response to a change in pH, and wherein the polymer gel network surrounds a tablet comprising a biologically active compound.

6. The pharmaceutical composition of claim 1, wherein the polymer gel network has a composition selected so that the polymer gel network is bioadhesive.

7. The pharmaceutical composition of any of claims 1-3, wherein the polymer gel network has a composition selected so that the pharmaceutical composition is suitable for ocular delivery.

8. The pharmaceutical composition of claim 7, wherein the polymer gel network is lightly crosslinked, and wherein the biologically active compound is selected from the group consisting of water and saline, so that the pharmaceutical composition functions as a lacrimator.

9. The pharmaceutical composition of claim 7, wherein the biologically active compound is selected from the group consisting of ophthalmic diagnostic agents, ophthalmic surgical additives, anti-glaucomals, anti-virals, anti-microbials.

10. The pharmaceutical composition of any of claims 1-3, wherein the polymer gel network has a composition selected so that the pharmaceutical composition is suitable for vaginal delivery.

11. The pharmaceutical composition of claim 10, wherein the polymer gel network has a composition selected so that the polymer gel network undergoes a volume change in response to a change in pH, and wherein the biologically active compound comprises an antifungal.

12. The pharmaceutical composition of claim 10, wherein the biologically active compound is selected from the group consisting of lubricants, spermicides, and virucides.

13. The pharmaceutical composition of any of claims 1-3, wherein the polymer gel network has a composition selected so that the pharmaceutical composition is suitable for rectal delivery.

14. The pharmaceutical composition of claim 13, having the form of a suppository.

15. The pharmaceutical composition of any of claims 1-3, wherein the polymer gel network has a composition selected so that the pharmaceutical composition is suitable for dermal delivery.

16. The pharmaceutical composition of claim 15, wherein the biologically active compound is present at an amount at least 20% by weight of the polymer gel network.

17. The pharmaceutical composition of claims 16, further comprising a backing

18. The pharmaceutical composition of claim 15, wherein the polymer gel network has a composition selected so that the polymer gel undergoes a change in volume in response to a change in the electric field, and the biologically active compound comprises a compound capable of transdermal delivery by iontophoresis, so that application of an electric field to the pharmaceutical composition results in simultaneous release of the biologically active compound from the polymer gel network and delivery of the biologically active compound by iontophoresis.

19. The pharmaceutical composition of any of claims 1-3, wherein the polymer gel network has a composition selected so that the pharmaceutical composition is suitable for internal delivery.

20. The pharmaceutical composition of claim 19, wherein the biologically active compound is selected from the group consisting of: insulin, hormones, and nitroglycerin.

21. The pharmaceutical composition of claim 1, wherein the polymer gel network comprises a first polymer that is a polysaccharide.

22. The pharmaceutical composition of claim 21, wherein the first polymer is a hydroxyalkyl-substituted polysaccharide.

23. The pharmaceutical composition of claim 21, wherein the first polymer is a cellulosic polymer.

24. The pharmaceutical composition of claim 21, wherein the first polymer is selected from the group consisting of: hydroxypropylcellulose, hydroxypropylmethylcellulose, starch, and hydroxyethylcellulose.

25. In a controlled-release pharmaceutical delivery composition, the improvement that comprises formulating the controlled-release composition from a polymer gel network having a known, acceptable toxilogical profile.

26. A device for controlled delivery of a biologically active compound to a biological environment, comprising:

a housing defining an inner space comprising a first compartment and a second compartment, the housing having an orifice allowing communication between the first compartment and an environment outside of the housing, the housing comprising a semi-permeable membrane that defines at least a portion of a side of the second compartment;

a moveable partition within the housing separating the first compartment from the second compartment; and

a polymer gel network having a known, acceptable toxilogical profile, the polymer network being disposed within the second compartment.

27. The device of claim 26 further comprising:

an effective amount of a biologically active compound disposed in the first compartment,

the device being arranged and constructed such that expansion of the polymer gel network in the second compartment exerts a mechanical force on the moveable partition so that the moveable partition moves in such a way that the second compartment is increased in volume and the first compartment is correspondingly

reduced in volume, so that the biologically active compound exits the device through the orifice.

28. The device off claim 26 or claim 27 wherein the polymer gel network has a composition selected so that the polymer gel network undergoes a volume change in response to a change in environmental condition.

29. The device of claim 28 wherein the change in environmental condition comprises a change in an environmental parameter selected from the group consisting of: temperature, pH, solvent composition; presence, energy, or orientation of an electric field; presence, energy, or orientation of a magnetic field; presence or magnitude of mechanical force; presence or energy of incident electromagnetic radiation.

30. In a device for controlled delivery of a biologically active compound to a biological environment, comprising:

a housing defining an inner space comprising a first compartment and a second compartment, the housing having an orifice allowing communication between the first compartment and the biological environment, the housing comprising a semi-permeable membrane that defines at least a portion of a side of the second compartment;

a moveable partition within the housing separating the first compartment from the second compartment; and

an expandable member disposed within the second compartment, so that expansion of the expandable member results in extrusion of the biologically active compound through the orifice, the improvement that comprises utilizing a polymer gel network having a known, acceptable toxilogical profile as the expandable member.

31. A device for controlled delivery of a biologically active compound into a biological environment, comprising:

a housing defining a compartment and having an orifice therein allowing communication between the compartment and the biological environment;

a biologically active compound disposed within the compartment and

an expandable member disposed within the compartment in such a way that expansion of the expandable member results in delivery of the biologically active compound to the biological environment by means of the orifice, the expandable member comprising a polymer gel network having a known, acceptable toxilogical profile.

32. In a device for controlled delivery of a biologically active compound into a biological environment, comprising:

a housing defining a compartment and having an orifice therein allowing communication between the compartment and the biological environment;

a biologically active compound disposed within the compartment and

an expandable member disposed within the compartment in such a way that expansion of the expandable member results in delivery of the biologically active compound to the biological environment by means of the orifice, the improvement that comprises utilizing a polymer gel network having a known, acceptable toxilogical profile as the expandable member.

33. A medical instrument, comprising:

a substrate material;

a polymer gel network disposed on at least a portion of a surface of the substrate material, the polymer gel network comprising a polyanion and a polycation interacting with one another to form a polyelectrolyte complex.

34. The medical instrument of claim 33, further comprising a biologically active compound loaded in the polymer gel network.

35. The medical instrument of claim 33 or 34, wherein the polymer gel network has a a composition selected so that the polymer gel network undergoes a volume change in response to a change in environmental condition.

36. The medical instrument of claim 35, wherein the change in environmental condition comprises a change in an environmental parameter selected from the group consisting of: temperature; pH; solvent composition; presence, energy, or orientation of an electric field; presence, energy, or orientation of a magnetic field; presence or magnitude of mechanical force; presence or energy of incident electromagnetic radiation.

37. The medical instrument of claim 33, wherein the polyanion comprises a polycarboxylic acid.

38. The medical instrument of claim 33 or claim 35, wherein the polycation comprises a polymer selected from the group consisting of: primary amines, secondary amines, tertiary amines, polyimines, and basic polypeptides.

39. The medical instrument of claim 38, wherein the poly cation is selected from the group consisting of: polyethyleneimine, polyethylenepiperazine, and collagen.

40. The medical instrument of claim 37, wherein the polycarboxylic acid is selected from the group consisting of: polyacrylic acid, polymethacrylic acid, and chondritin-6-sulfate.

41. A medical instrument, comprising:

a substrate material;

a polymer gel network disposed on at least a portion of a surface of the substrate material;

a biologically active compound loaded into the polymer gel network; and

an encapsulating means trapping the biologically active compound within the polymer gel network.

42. The medical instrument of claim 41, wherein the encapsulating means comprises a polymer that interacts with the polymer gel network to trap the biologically active compound within the polymer gel network.

43. The medical instrument of claim 41, wherein the substrate material is expandable and the encapsulating means is sensitive to mechanical stress, so that expansion of the substrate material results in release of the biologically active compound.

44. The medical instrument of claim 43, wherein the substrate material comprises a polyanion and the encapsulating means comprises a proton acceptor polymer.

45. The medical instrument of claim 44, wherein the proton acceptor polymer comprises polyethyleneglycol, polyethylene oxide, polypropylene glycol, polypropylene oxide, or combinations thereof.

46. The medical instrument of claim 42, wherein the substrate material comprises a polyamide material that has been functionalized with carboxylic acid groups.

45. The medical instrument of claim 44, wherein the proton acceptor polymer comprises polyethyleneglycol, polyethylene oxide, polypropylene glycol, polypropylene oxide, or combinations thereof.

46. The medical instrument of claim 44, wherein the proton acceptor polymer comprises poly (N,N-dimethylacrylamide).

47. The medical instrument of claim 44, wherein the substrate material comprises a polyamide material that has been functionalized with carboxylic acid groups.

48. The medical instrument of claim 47, wherein the polyamide material has been functionalized by treatment with a di- or multi-functional polyisocyanate, followed by treatment with a polycarboxylic acid.

49. The medical device of claim 44, wherein the substrate material forms an angioplasty balloon.

50. A method, comprising:

providing a medical instrument;

coating at least a portion of a surface of the medical instrument with a polymer gel network comprising a polyanion and a polyanion and a polycation interacting with one another to form a polyelectrolyte complex.

51. A method, comprising:

providing a medial instrument having coated on at least a portion of a surface thereof a polymer gel network comprising a polyanion and a polycation interacting with one another to form a polyelectrolyte complex; and

loading a biologically active material into the polymer gel network.

52. A method, comprising:

providing a medical instrument having coated on at least a portion of a surface thereof a polymer gel network comprising a polyanion and a polycation interacting with one another to form a polyelectrolyte complex, the polymer gel network having loaded therein a biologically active compound;

positioning the medical instrument in a biological environment; and

delivering the biologically active compound into the biological environment.

53. A method, comprising:

providing a medical instrument having a polymer gel network coated on at least a portion of a surface;

loading a biologically active material into the polymer gel network; and

encapsulating the biologically active material within the polymer gel network.

54. The method of claim 53, wherein the step of encapsulating comprises providing a polymer that interacts with the polymer gel network and traps the biologically active compound therein.

55. A method, comprising:

providing an expandable medical instrument having a polymer gel network coated on at least a portion thereof, the polymer gel network having a biologically active compound trapped by means of an encapsulator, the encapsulator being sensitive to mechanical force;

positioning the expandable medical instrument in a biological environment; and

expanding the medical instrument so that mechanical force is exerted on the encapsulator and the biologically active compound is delivered in to the biological environment.

56. A crosslinked, responsive polymer gel network comprising polymer chains interconnected by way of a multifunctional crosslinker, wherein the polymer chains and the crosslinker have a known acceptable toxicological profile.

57. A crosslinked, responsive polymer gel network comprising polymer chains interconnected by way of crosslinkages, wherein each of the said polymer chains and crosslinkages has a known acceptable toxicological profile.

58 A crosslinked, responsive polymer gel network comprising polymer chains interconnected by way of crosslinkages, the polymers and crosslinkages each having a known acceptable toxicological profile and each being obtainable from a precursor that is used in a process for making a material that has a known acceptable toxicological profile.

59. The responsive polymer gel network of any one of claims 56-58, wherein a leachate from said network has an acceptable toxicological profile.

60. The responsive polymer gel network of any one of claims 56-58, wherein the network contains residual elements that have an acceptable toxicological profile.

61. The responsive polymer gel network of any one of claims 56-58, wherein said polymer network further comprises a solvent that has a known acceptable toxicological profile.

62. A crosslinked, responsive polymer gel network comprising polymer chains derived from a natural product polymer, said polymer chains interconnected by way of a multifunctional crosslinker, wherein the polymer chains and crosslinker have a known acceptable toxicological profile.

63. A crosslinked, responsive polymer gel network comprising polymer chains derived from a natural product polymer, said polymer chains interconnected by way of crosslinkages, wherein said crosslinkages have a known acceptable toxicological profile.

64. A crosslinked, responsive polymer gel network comprising polymer chains derived from a natural product polymer, the polymer chains interconnected by way of crosslinkages, the polymers and crosslinkages each having a known acceptable toxicological profile and each being obtainable from a precursor that is used in a process for making a material that has a known acceptable toxicological profile.

65. The responsive polymer gel network of any one of claims 62-64, wherein a leachate from said network has an acceptable toxicological profile.

66. The responsive polymer gel network of any one of claims 62-64, wherein the network contains residual elements that have an acceptable toxicological profile.

67. The responsive polymer gel network of any one of claims 62-64, wherein said polymer network further comprises a solvent that has a known acceptable toxicological profile.

68. A crosslinked, responsive polymer gel network comprising polysaccharide polymer chains interconnected by way of a multifunctional crosslinker, wherein the polysaccharide polymer chains and crosslinker have a known acceptable toxicological profile.

69. A crosslinked, responsive polymer gel network comprising polysaccharide polymer chains interconnected by way of crosslinkages, wherein the crosslinkages have a known acceptable toxicological profile.

70. A crosslinked, responsive polymer gel network comprising polysaccharide polymer chains interconnected by way of crosslinkages, the polysaccharide polymer chains and crosslinkage each having a known acceptable toxicological profile and each being obtainable from a precursor that is used in a process for making a material that has a known acceptable toxicological profile.

71. The responsive polymer gel network of any one of claims 68-70, wherein a leachate from said network has an acceptable toxicological profile.

72. The responsive polymer gel network of any one of claims 68-70, wherein the network contains residual elements that have an acceptable toxicological profile.

73. The responsive polymer gel network of any one of claims 68-70, wherein said polymer network further comprises a solvent that has a known acceptable toxicological profile.

74. A crosslinked, responsive polymer gel network comprising starch polymer chains interconnected by way of multifunctional crosslinker, wherein the starch polymer chains and crosslinker have a known acceptable toxicological profile.

75. A crosslinked, responsive polymer gel network comprising starch polymer chains interconnected by way of crosslinkages, wherein said crosslinkages have a known acceptable toxicological profile.

76. A crosslinked, responsive polymer gel network comprising starch polymer chains interconnected by crosslinkages, the starch polymer chains and crosslinkage each having a known acceptable toxicological profile and each being obtainable from a precursor that is used in a process for making a material that has a known acceptable toxicological profile.

77. A crosslinked, responsive polymer gel network comprising cellulose ether polymer chains interconnected by way of multifunctional crosslinker, wherein the cellulose ether polymer chains and crosslinker have a known acceptable toxicological profile.

78. A crosslinked, responsive polymer gel network comprising cellulose ether polymer chains interconnected by way of a plurality of crosslinkages, wherein the crosslinkages have a known acceptable toxicological profile.

79. A crosslinked, responsive polymer gel network comprising cellulose ether polymer chains interconnected by way crosslinkages, the cellulose ether polymer chains and crosslinkages each having a known acceptable toxicological profile and each being obtainable from a precursor that is used in a process for making a material that has a known acceptable toxicological profile.

80. The polymer gel network of any one of claims 77-79, wherein the cellulose ether is selected from the group consisting of hydroxyethylcellulose, hydroxypropylcellulose, and hydroxypropylmethylcellulose.

81. The polymer gel network of any one of claims 56, 62, 68, 74 or 77, wherein the polymer chains are crosslinked with a multifunctional carboxylic acid.

82. The polymer gel network of any one of claim 26, wherein the multifunctional carboxylic acid is selected from the group consisting of adipic acid, sebacic acid, succinic acid, citric acid, 1,2,3,4- butanetetracarboxylic acid, and 1,10 decanedicarboxylic acid.

83. The responsive polymer gel network of any one of claims 57, 58, 63, 64, 69, 70, 75, 76, 78, or 79, wherein the crosslinkage is a multifunctional carboxylic acid and the crosslinker is an acyl halide derivative of said acid.

84. The responsive polymer gel network of any one of claim 83, wherein the acyl halide derivative of a multifunctional carboxylic acid is selected from the group consisting of the acyl halide derivative of adipic acid, sebacic acid, succinic acid, 1,2,3,4- butanetetracarboxylic acid, and 1,10 decanedicarboxylic acid.

85. The polymer gel network of any one of claims 56, 59, 68, 70 or 74-79, wherein the gel is pH-responsive.

86. The polymer gel network of claim 85, wherein said pH-response is triggered by a change in an environmental condition to which the gel is exposed, wherein the change is selected from the group consisting of a change in temperature, a change in ion concentration, a change in solvent concentration, and a change in electric field.

87. The polymer gel network of any one of claims 56-58, 62-64, 68-70, or 75-79, further comprising a ligand immobilized to the network.

88. The polymer network of claim 87, wherein the ligand has a known acceptable toxicological profile.

89. A method for making a crosslinked polymer network for a particular use, comprising:

selecting a polymeric starting material capable of being crosslinked, wherein the polymeric starting material selected for the particular use has a known acceptable toxicological profile for the particular use or for a related use;

selecting a crosslinker capable of crosslinking the polymeric starting material, wherein the crosslinker selected for the particular use has a known acceptable toxicological profile for the particular use or for a related use;

contacting the crosslinker and polymeric starting material under conditions sufficient to form the three-dimensional, crosslinked polymer network.

90. A method for making a crosslinked polymer gel network for a particular use, comprising:

selecting a polymeric starting material capable of being crosslinked, wherein the polymeric starting material selected for the particular use has a known acceptable toxicological profile for the particular use or for a related use;

selecting a crosslinker capable of crosslinking the polymeric starting material, wherein the network, after formation thereof, contains a crosslinkage that has a known acceptable toxicological profile;

contacting the crosslinker and polymeric starting material under conditions sufficient to form the polymer gel network containing the crosslinkage.

91. A method of making a polymer gel network, comprising:

contacting a crosslinker comprising an acyl halide derivative of a multifunctional carboxylic acid with a polysaccharide under conditions sufficient for a three-dimensional, polymer gel network to form, the gel network comprising polysaccharide chains crosslinked with the acid.

92. The method of claim 91, wherein the contacting step comprises contacting with a polysaccharide that is starch.

93. The method of claim 91, wherein the contacting step comprises contacting with a polysaccharide that is a cellulose ether.

94. The method of claim 89, wherein the contacting step comprises contacting with a crosslinker that is an acyl chloride derivative of a multifunctional carboxylic acid selected from the group consisting of an acyl halide derivative of adipic acid, sebacic acid, succinic acid, 1,2,3,4- butanetetracarboxylic acid, and 1,10 decanedicarboxylic acid.

95. The method of claim 89, wherein the contacting step comprises contacting with a crosslinker that is a multifunctional carboxylic acid.

96. The method of claim 95, wherein the multifunctional carboxylic acid is selected from the group consisting of adipic acid, sebacic acid, succinic acid, citric acid, 1,2,3,4- butanetetracarboxylic acid, and 1,10 decanedicarboxylic acid.

97. The method of claim 90, wherein the contacting step comprises contacting with a crosslinker that is an acyl halide derivative of a multifunctional carboxylic acid.

98. The method of claim 97, wherein the acyl halide derivative of a multifunctional carboxylic acid is selected from the group consisting of an acyl halide derivative of adipic acid, sebacic acid, succinic acid, 1,2,3,4- butanetetracarboxylic acid, and 1,10 decanedicarboxylic acid.

99. The method of claim 95, wherein the contacting step comprises contacting with a cellulose ether selected from the group consisting of hydroxyethylcellulose, hydroxypropylcellulose, and hydroxpropylmethylcellulose.

100. A method of separating a desired substance from a solution, comprising:

introducing a polymer gel network of any one of claims 56-58, 62-64, 68-70, or 74-79, capable of incorporating said substance from a solution containing the substance;

inducing a volumetric change of the gel by changing an environmental condition to which the gel is exposed so that the gel incorporates the substance and separates the substance from the solution.

101. A method of separating a desired substance from a solution, comprising:

introducing a polymer gel network of any one of claims 56-58, 62-64, 68-70, or 74-79, capable of excluding said substance from a solution containing the substance;

inducing a volumetric change of the gel by changing an environmental condition to which the gel is exposed so that the gel excludes the substance and separates the substance from the solution.

102. A method of delivering a substance into an environment of use, comprising:

incorporating the substance into a polymer gel network of any one of claims 56-58, 62-64, 68-70, or 74-79;

inducing a volumetric change in the network by changing an environmental condition to which the network is exposed so that the network disgorges the substance to the environment of use.

103. A method for controlling a reaction in an environment containing a reactant, comprising:

introducing into the environment the polymer gel network of claim 87 containing a ligand reactive with the reactant when the ligand is exposed to the reactant;

changing an environmental condition of the network to cause a volumetric change therein and expose the ligand to the reactant, thereby controlling the reaction.

104. A method for removing a substance from an environment containing a the substance, comprising;

introducing into the environment the polymer gel network of claim 87 containing a ligand reactive with the substance when the ligand is exposed to the substance;

changing an environmental condition of the network to cause a volumetric change therein and expose the ligand to the substance, thereby incorporating the substance into the gel network and removing the substance from the environment.

105. A cosmetic composition comprising a polymer network of claim 56.

106. A wound dressing comprising a polymer network of claim 56.

107. A pharmaceutical composition comprising a pharmaceutically effective amount of a biologically active compound and a polymer network of claim 56.

108. An iontophoretic device comprising the polymer network of claim 56.

109. A monitoring electrode comprising the polymer network of claim 56.

110. An adhesive device comprising the polymer network of claim 56

111. A method of loading a solute into a crosslinked polymer gel network, comprising:

contacting a solution of the solute with a polymer gel network of claim 56nd loading polymer, under conditions sufficient for the solute to selectively partition into the polymer gel network.

112. The method of claim 111, wherein the step of contacting comprises contacting a polymer gel that is more hydrophobic than the loading polymer.

113. The method of claim 111, wherein the step of contacting comprises contacting a polymer gel that is less hydrophobic than the loading polymer.

114. The method of claim 111, further comprising the step of contacting the solution with a salt.

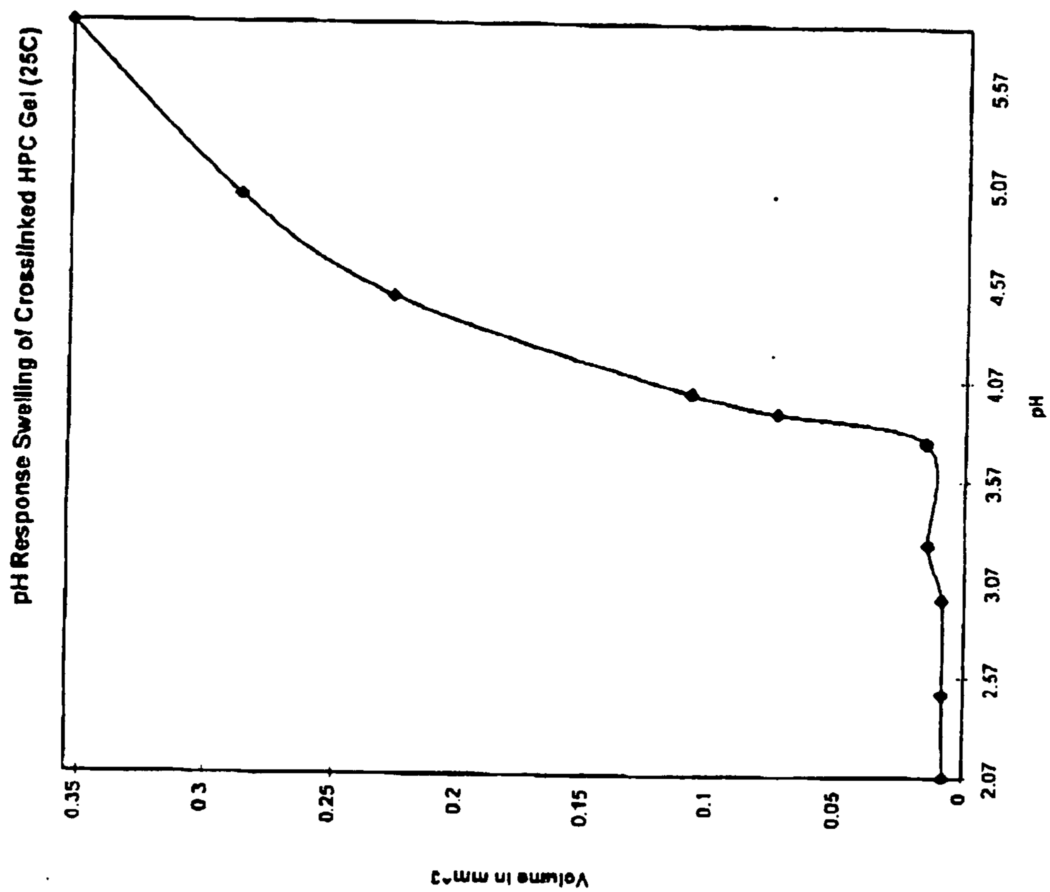
pH Response Swelling of Crosslinked HPC Gel (25C)
Volume in mm^3
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
pH
2.07
2.57
3.07
3.57
4.07
4.57
5.07
5.57

FIG. 1

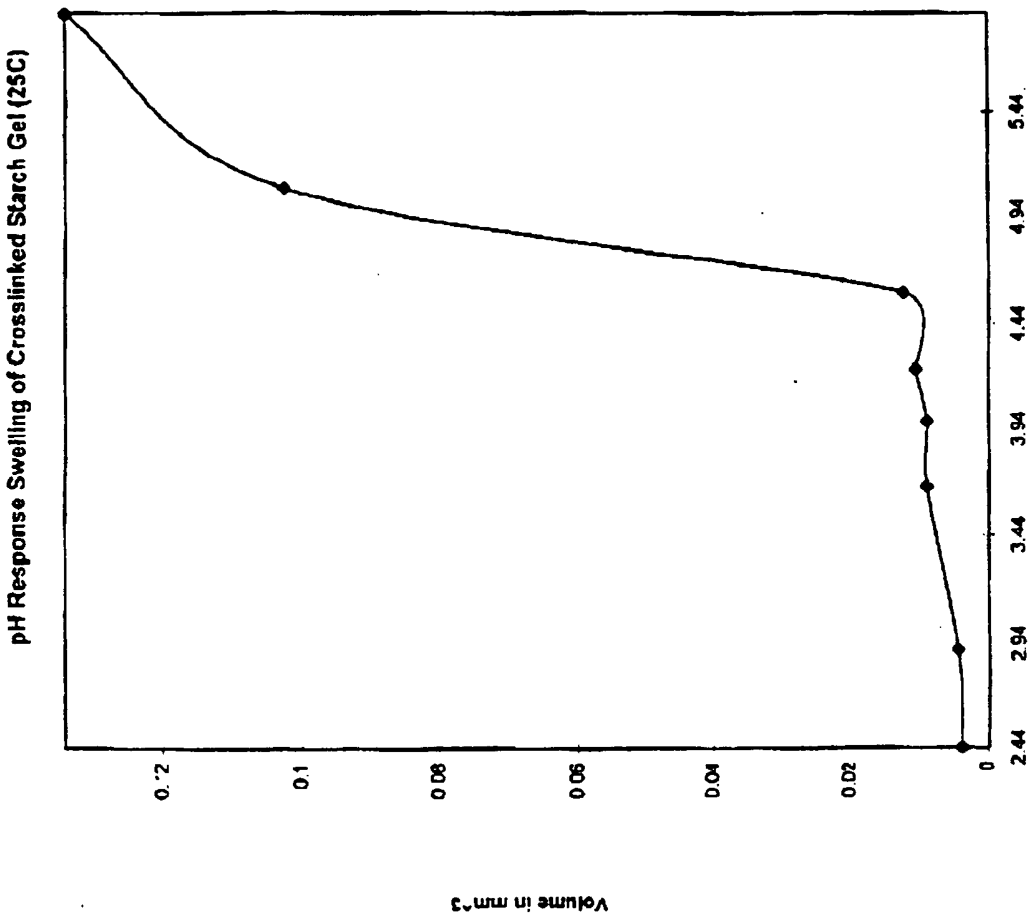
pH Response Swelling of Crosslinked Starch Gel (25C)
Volume in mm^3
pH
2.44
2.94
3.44
3.94
4.44
4.94
5.44
0
0.02
0.04
0.06
0.08
0.1
0.12

FIG. 2

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☑ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☑ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: ______________________

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.